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# cancer treatment reports

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*The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.*

# Proceedings of the Symposium on Antiestrogen Therapy for Hormone-Dependent Tumors

Editor: Marc Lippman

September 29, 1979
Sorrento, Italy

# Estrogen-Receptor Binding and Biologic Activity of Tamoxifen and Its Metabolites

A. E. Wakeling* and S. R. Slater[1]

## SUMMARY

**In female patients treated with tamoxifen (T), the major metabolite of T in serum is *N*-desmethyltamoxifen (*N*-d-Me T). The serum concentration of d-Me T may equal or exceed that of T, and 4-hydroxytamoxifen (4-OH T) is present in much lower concentration (< 10% that of T). The biologic properties and estrogen-receptor binding affinity of T and its desmethyl and 4-hydr- -metabolites have been compared. In competition studies with the rat uterus estrogen receptor at 0°C and 25°C, the relative affinities of T and d-Me T were similar, and their apparent affinity decreased when the incubation temperature was increased (38.2, 21.0 and 1.8, 1.1, respectively at 0°C and 25°C; 17β-estradiol = 100). In contrast, 4-OH T was a more potent inhibitor of estradiol binding (110 and 188 at 0°C and 25°C) and its apparent affinity increased, rather than decreased, when the incubation temperature was raised from 0°C to 25°C. These differences in receptor binding were not reflected in biologic activity; all three compounds were potent antiestrogens of approximately equal activity in the rat. In patients, the pharmacologic effect of T may be due, in part, to d-Me T, but it is unlikely that 4-OH T plays a major role because of its relatively low serum concentration. The antiestrogenic potencies of T, d-Me T, and 4-OH T are similar, so the extent of metabolism in individual patients is unlikely to influence clinical response to tamoxifen therapy.**

[Cancer Treat Rep 64:741–744, 1980]

The nonsteroidal antiestrogen tamoxifen (T) (Nolvadex, property of Imperial Chemical Industries, Ltd) is widely used in therapy for advanced breast cancer. There is a great deal of current interest in the metabolism of this compound in the human subject and in the question of what role, if any, the metabolites of T may have in the biologic action of the drug. The original studies of T metabolism in animals (1) and in female patients (2) identified 4-hydroxytamoxifen (4-OH T) as a major metabolite. Studies by Jordan et al (3) suggested that 4-OH T is a potent antiestrogen, which contributes to the antiestrogenic effect observed in patients treated with T. More recently, it has been shown (4) that, in female patients treated with T, the major metabolite of T in serum is desmethyltamoxifen (d-Me T) and not 4-OH T as previously thought. The structures of T, d-Me T, and 4-OH T are shown in figure 1. We have conducted a series of experiments to compare the biologic properties of these compounds and their interaction with estrogen receptors. We have also examined the hypothesis that the kinetics of interaction between estrogens (and antiestrogens) and the cytoplasmic estrogen receptor determines biologic response (5,6). In competition studies, potent estrogens behave like estradiol while weak agonists and antagonists are characterized by a shift to apparent lower binding affinity when the temperature or time of incubation is increased.

## MATERIALS AND METHODS

Cytosol was prepared from mature rat uterus by homogenization in 10 mM phosphate buffer, pH 7.4,

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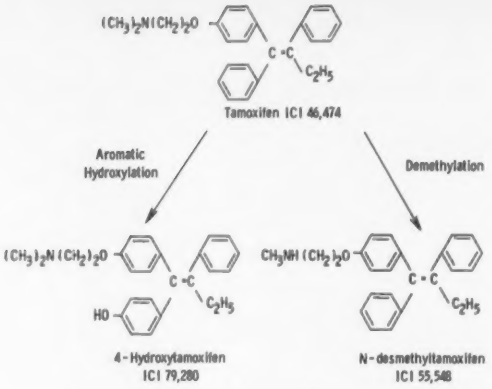


FIGURE 1.—Metabolism of T; structures of T, d-Me T, and 4-OH T.

containing 1.5 mM EDTA, 0.1% bovine serum albumin, and 2 mM β-mercaptoethanol at 4°C, and centrifugation at 100,000 *g* for 1 hour at 0°C. Aliquots of cytosol (100 μl) were incubated with [2,-4,6,7-$^3$H]estradiol-17β ($5 \times 10^{-9}$ M, 85–110 curies/mmol; Radiochemical Centre, Amersham, England), in the absence or presence of increasing concentrations of estradiol-17β, T, d-Me T, or 4-OH T (100 μl) for 2 hours at 0°C or 25°C. After incubation, a suspension of 100 μl of dextran-coated charcoal (0.5% dextran T-70, 5% Norit A charcoal wt/vol in phosphate buffer) was added and incubation was continued for 15 minutes at 0°C. After removal of charcoal by centrifugation, protein-bound $^3$H-estradiol in the supernatant (200 μl) was measured by liquid scintillation counting in 4 ml of PCS 60 (Amersham) scintillation fluid. Relative binding affinities (RBAs) were calculated as the ratio of concentrations required to reduce $^3$H-estradiol-specific binding by 50% (estradiol-17B designated 100 at 0°C and 25°C).

Antiestrogenic activity was determined by measuring the inhibition of implantation in pregnant rats given a single dose of test compound at 4 PM on Day 4 of pregnancy as described by Harper and Walpole (7).

## RESULTS

The competition of T, d-Me T, and 4-OH T with $^3$H-estradiol for cytoplasmic estrogen receptors of rat uterus is shown in figures 2–4, respectively.

In all cases, nonradioactive estradiol competed with $^3$H-estradiol to give a classic competition curve that was identical at both 0°C and 25°C, suggesting that equilibrium was rapidly established. At 0°C, 4-OH T behaved very much like estradiol (RBA of 109.8), while T and d-Me T demonstrated lower affinities for the receptor (RBAs of 38.2 and 21.0, respectively). When the temperature was raised to 25°C, the apparent affinity of 4-OH T increased (RBA of 187.8), while the curves for T and d-Me T shifted dramatically to apparent affinities (RBAs of 1.8 and 1.1, respectively) much less than those observed at 0°C. This shift in the T competition curve reflects an increased dissociation rate constant of T in comparison to estradiol (8).

We compared the antiestrogenic potency of T, d-Me T, and 4-OH T by measuring their efficacy in terminating early pregnancy in the rat (7). When administered on the evening of Day 4 of pregnancy, antiestrogens prevent implantation by antagonizing the preimplantation surge of estradiol. The data in figure 5 show that all compounds have very similar antiestrogenic potency in this test; the minimal fully effective dose was in the range of 0.05–0.1 mg/kg for all three compounds.

## DISCUSSION

The hypothesis introduced by Bouton and Raynaud (5,6) suggests that the reduced RBA values observed with T and d-Me T, when the incubation temperature was raised from 0°C to 25°C, characterizes these compounds as antagonists (antiestrogens). In contrast, the receptor binding characteristics of 4-OH T were those expected of a potent agonist (estrogen). It is apparent that both metabolites of T are potent antiestrogens and that the differences in receptor-binding kinetics between T, d-Me T, and 4-OH T were not reflected in the biologic activity of these substances. Thus, receptor-binding kinetics did not reliably indicate biologic activity. These results agree with those of other recent studies of T and 4-OH T binding to estrogen receptors of calf uterus (9) and of rat uterus and mammary tumors (10).

In female patients treated with T, serum concentrations of d-Me T may equal or exceed those of T, but the concentration of 4-OH T is very low (< 10% T) (11). Since the biochemical and biologic properties of T and d-Me T are very similar and their serum concentrations in patients are comparable, it is possible that the d-Me T metabolite plays an important role in inducing the regression of estrogen-dependent mammary carcinoma. However, it should be emphasized that, clinically, the degree of metabolism of T to d-Me T will not affect patient response. Finally, because of the low free serum concentration of 4-OH T, it is less likely to play a significant role in inducing tumor regression in patients treated with T.

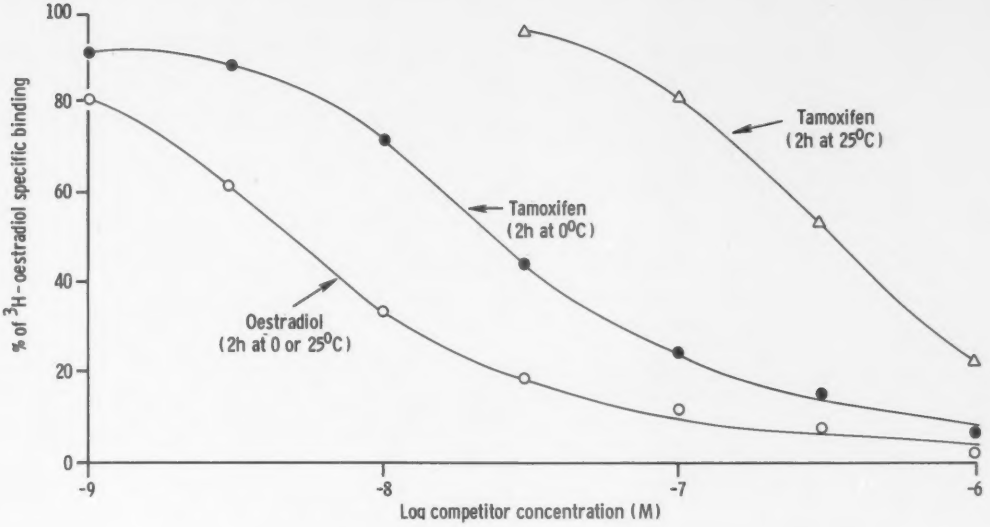


FIGURE 2.—Competition of T for rat uterus estrogen receptor.

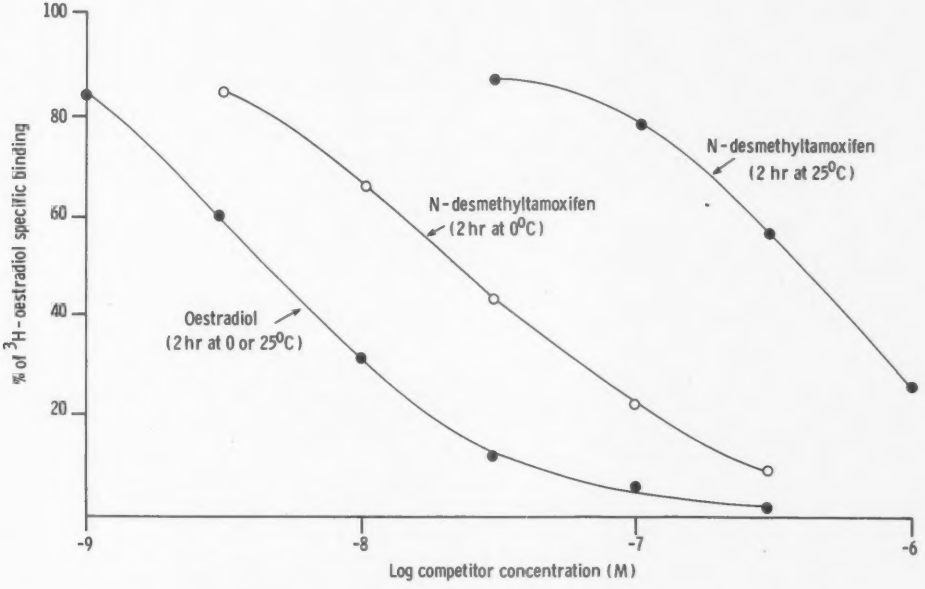


FIGURE 3.—Competition of *N*-d-Me T for rat uterus receptor.

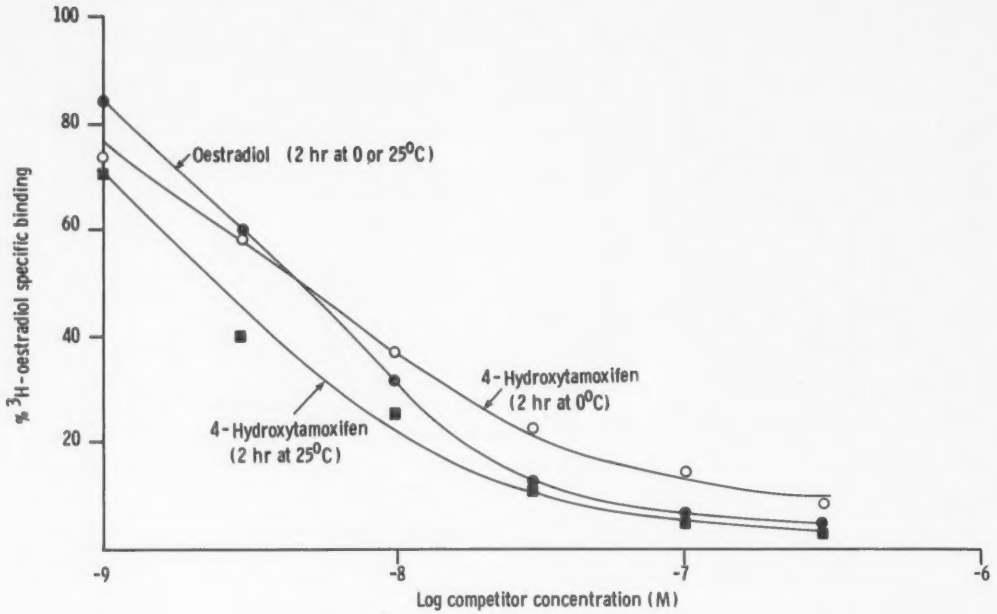


FIGURE 4.—Competition of 4-OH T for rat uterus receptor.

| | Dose* (mg/kg) | No. pregnant/No. mated | % Egg loss |
|---|---|---|---|
| Tamoxifen ICI 46,474 | 0.2 | 0/4 | 100 |
| | 0.1 | 0/4 | 100 |
| | 0.05 | 0/4 | 100 |
| | 0.025 | 3/4 | 30 |
| 4-Hydroxytamoxifen ICI 79,280 | 0.2 | 0/5 | 100 |
| | 0.1 | 0/4 | 100 |
| | 0.05 | 1/4 | 96 |
| | 0.025 | 4/4 | 0 |
| Desmethyltamoxifen ICI 55,548 | 0.2 | 0/4 | 100 |
| | 0.1 | 0/4 | 100 |
| | 0.05 | 1/3 | 98 |
| | 0.025 | 4/4 | 30 |

FIGURE 5.—Prevention of implantation in rats by T, 4-OH T, and *N*-d-Me T. * = single oral dose at 4 PM, Day 4 of pregnancy.

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# Pharmacology of Tamoxifen in Laboratory Animals[1]

V. Craig Jordan,[2,*] Karen E. Allen, and C. J. Dix[3]

## SUMMARY

**This paper reviews the recent laboratory findings about the nonsteroidal antiestrogen, tamoxifen, and its more potent major metabolite, monohydroxytamoxifen. Both compounds stimulate progesterone receptor synthesis in the rat uterus, and there is an inhibition of cell division in the uterine luminal epithelial cells. The effects of tamoxifen in vivo may be a result of the net effects of the parent compound and monohydroxytamoxifen. In rats with dimethylbenzanthracene (DMBA)-induced rat mammary carcinomata, young tumors that are estrogen receptor- and progesterone receptor-rich respond more favorably to tamoxifen than do older estrogen receptor- and progesterone receptor-poor tumors. However, the antitumor effect of tamoxifen in the DMBA-induced rat mammary carcinoma model is probably a result of the blockade of tumor estrogen receptors, a reduction in circulating gonadotropins, lower circulating estrogen levels, and lower circulating prolactin levels. The 30-days treatment of rats with tamoxifen 30 days after DMBA resulted in a dose-related decrease in the appearance and numbers of mammary tumors; however, only continuous therapy maintained animals in a tumor-free state. Monohydroxytamoxifen was a less-potent antitumor agent, probably because it is cleared from the rat more rapidly than tamoxifen. The present laboratory findings support the clinical use of tamoxifen as a treatment of endometrial carcinoma and the resultant metastases and as an adjuvant therapy after surgery for breast cancer.**

[Cancer Treat Rep 64:745–759, 1980]

Harper and Walpole (1,2) were the first to compare the antiestrogenic properties of tamoxifen with those of its estrogenic *cis* isomer, ICI 47,699. Since their study, tamoxifen has been shown to be of value in the treatment of advanced breast cancer (3,4); however, the increasing clinical use of antiestrogens has stimulated laboratory efforts to determine the mechanism of action. The fruits of such research will obviously provide an understanding of estrogen-stimulated growth, but perhaps of greater significance will be the wealth of new biochemical data generated, which can be exploited to design novel pharmacologic agents.

The aim of this paper is to update the published data on the pharmacology of tamoxifen, and to consider various interesting aspects of the drug that could be exploited in the clinic. One important pharmacologic finding since the first tamoxifen workshop in Miami (5) has been that one of tamoxifen's metabolites, monohydroxytamoxifen (6), has potent antiestrogenic properties (7). We compared the pharmacology of the parent compound to its major metabolite in several experiments.

This paper is divided into four major sections. The first part of the paper focuses on the estrogenic and antiestrogenic properties of tamoxifen and its

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[2]Ludwig Institute for Cancer Research, Inselspital, Bern, Switzerland.

[3]The synthesis of tamoxifen derivatives by Dr. Dora Richardson and Dr. E. R. Clark, and the assay of tamoxifen in the LMCI tumor system by Dr. B. Dixon, are gratefully acknowledged.

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metabolites. The antitumor properties of tamoxifen are described in transplantable and dimethylbenzanthracene (DMBA)-induced rat mammary tumors and compared with the antitumor properties of monohydroxytamoxifen. Finally, the clinical relevance of the laboratory findings is discussed with a view to supporting the newer approaches of cancer therapy with antiestrogen.

## ANTIESTROGENIC PROPERTIES OF TAMOXIFEN AND ITS METABOLITES

In the rat, tamoxifen is a partial estrogen agonist with antiestrogenic properites (2); however, there are important species differences qualifying whether tamoxifen is an estrogen or an antiestrogen. In the mouse, tamoxifen is predominantly estrogenic (1,8), although a few studies have documented antiestrogenic effects (9,10). In the chick, tamoxifen appears to be a pure antiestrogen (11). In man, tamoxifen seems to be antiestrogenic. This review focuses on the biologic effect of tamoxifen in the rat.

The metabolism of tamoxifen in a variety of laboratory animals has been described by Fromson and co-workers (6). In the rat, the major nonconjugated metabolite is monohydroxytamoxifen (metabolite B), which is subsequently hydroxylated to dihydroxytamoxifen (metabolite D) (fig 1). Both of these metabolites are antiestrogenic in the rat (7); however, the finding that monohydroxytamoxifen is a more potent antiestrogen than the parent compound stimulated an investigation of the metabolite's biologic properties. Figure 2 shows an experiment in which the increased potency of monohydroxytamoxifen was compared with that of tamoxifen in the 3-day, immature rat uterine weight test. This study raised the question of the role of the metabolite in the mechanism of action of tamoxifen as an antiestrogen and antitumor agent. Ferguson and Katzenellenbogen (13) recently suggested that nonsteroidal antiestrogens are pro-drugs which are converted into more active polar metabolites before binding within estrogen target tissues (14). In support of the concept that the parent compounds have inherent biologic activity, nonsteroidal antiestrogens including tamoxifen can control the growth of MCF 7 human breast cancer cells in long-term tissue culture (15) under conditions where no metabolic changes can be detected (16).

To address the role of metabolic activation, we tested the antiestrogenic activity of a series of substituted (F, Cl, and $-CH_3$) derivatives of tamoxifen that do not undergo metabolic conversion to monohydroxytamoxifen. All compounds have only about one tenth the potency of tamoxifen as antiestrogens in the 3-day, immature rat uterine weight test, but the compounds are equipotent with tamoxifen in their ability to inhibit the binding of [$^3$H] estradiol to the rat uterine estrogen receptor in vitro. These results suggest that, over the range of the partial-agonist dose-response curve, the biologic activity of tamoxifen is the net result of the activities of the parent compound and its metabolites. Since the derivatives of tamoxifen are all antiestrogenic, it seems that the metabolic activation of nonsteroidal antiestrogens is only an advantage rather than a requirement for activity (17). Recently, Rochefort (personal communication) has found that the administration of [$^3$H] tamoxifen to rats results in the accumulation of [$^3$H] monohydroxytamoxifen in the uterine nuclei. These findings support the concept of metabolic activation in vivo.

The role of metabolism in the mechanism of action of tamoxifen depends on two major factors: first, the capacity of the liver to metabolize the drug to active metabolites, and second, the main metabolic pathways in the species under study. In the rat, low doses of tamoxifen produce proportionally higher concentrations of active metabolites, which, in turn, elicit biologic effects (7). At very high doses, tamoxifen becomes the predominant ligand for receptor binding. With regard to the second factor, it has recently been suggested (18) that, in the human, the primary metabolic route for tamoxifen is via *N*-demethylation to desmethyltamoxifen, and not hydroxylation to monohydroxytamoxifen. It is not known whether desmethyltamoxifen is then hydroxylated (which would provide a pool of potential high-affinity antiestrogenic ligands). Monohydroxytamoxifen has been detected in breast cancer patients receiving tamoxifen therapy, but further study of the metabolism of tamoxifen is necessary before the contribution of metabolic activation of tamoxifen can be evaluated in man.

In the first series of experiments, we considered the consequences of the action of estradiol, tamoxifen, and monohydroxytamoxifen in the immature rat uterus. The administration of various doses of estradiol benzoate (0.025–25 μg) to immature rats resulted in a dose-related increase in uterine wet weight, determined at 48 hours. Maximal rises were observed with 2.5 μg estradiol benzoate (fig 3). Monohydroxytamoxifen increased uterine wet weight to only 30% of that attained by estradiol benzoate. Tamoxifen was also a partial agonist, but less active than monohydroxytamoxifen, at lower doses.

Histologic examination of the luminal epithelial cells demonstrated that the two antiestrogens were only partially active in causing cell division (fig 4). The dose of estradiol benzoate that produced a max-

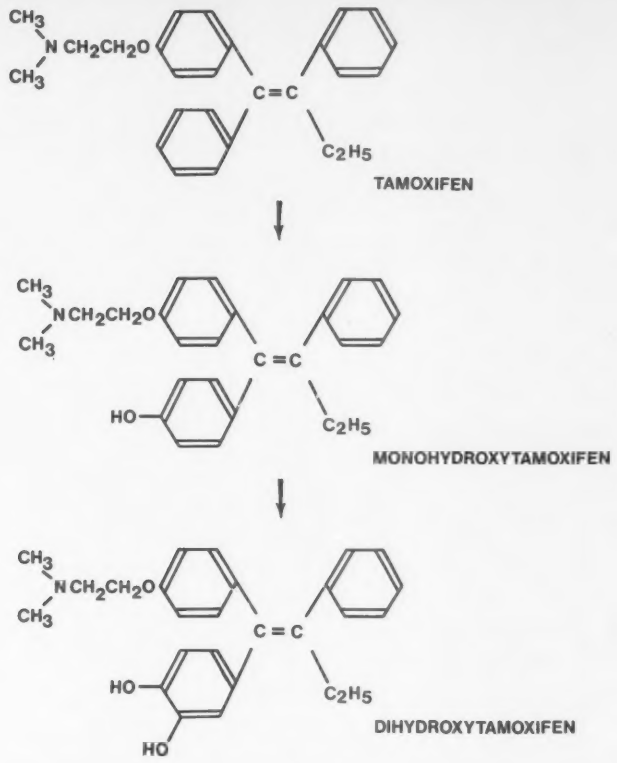


FIGURE 1.—Suggested pathway for the oxidative metabolism of tamoxifen in the rat (ref 6).

imal increase in uterine wet weight produced an enormous increase in mitotic activity; 80% of the luminal epithelial cells underwent mitosis between 41 and 48 hours. This result contrasted markedly with the maximal mitotic activity of 10% of the epithelial cells after antiestrogen. The apparent decrease in mitotic activity in the 25-μg estradiol benzoate group was the result of cells coming into mitosis earlier than 41 hours (the time of colchicine administration), rather than a suppression of mitotic activity by large doses of estrogen (unpublished observation).

## EFFECT OF TAMOXIFEN AND MONOHYDROXYTAMOXIFEN ON HORMONE RECEPTOR SYSTEMS

Since Jensen and Jacobson (20) first demonstrated that the administration of [$^3$H] estradiol to immature rats results in the localization and retention of radioactivity in estrogen target tissues (ie, uterus and vagina), interest has focused on the subcellular control of estrogen-dependent events. Estradiol is believed to stimulate uterine growth by binding to cytoplasmic receptors, which then translocate to the cell nucleus (21) where the complex increases RNA polymerase activity. As a result, uterine RNA and protein levels increase (22), and the depleted cytoplasmic estrogen receptors are replenished by resynthesis (23). Cell division occurs 24–48 hours after the initial stimulus.

The administration of increasing doses of tamoxifen or monohydroxytamoxifen to immature rats results in a dose-related decrease in the binding of [$^3$H] estradiol to the uterine cytoplasmic estrogen receptors, whether the determinations are undertaken in vitro (12) or in vivo (24).

A direct comparison of the inhibition of [$^3$H] estradiol binding to rat uterine estrogen receptors in vitro (fig 5) demonstrates the increased affinity of monohydroxytamoxifen for the receptor. The free phenolic hydroxyl group is extremely important for high-affinity binding, because the methoxy deriv-

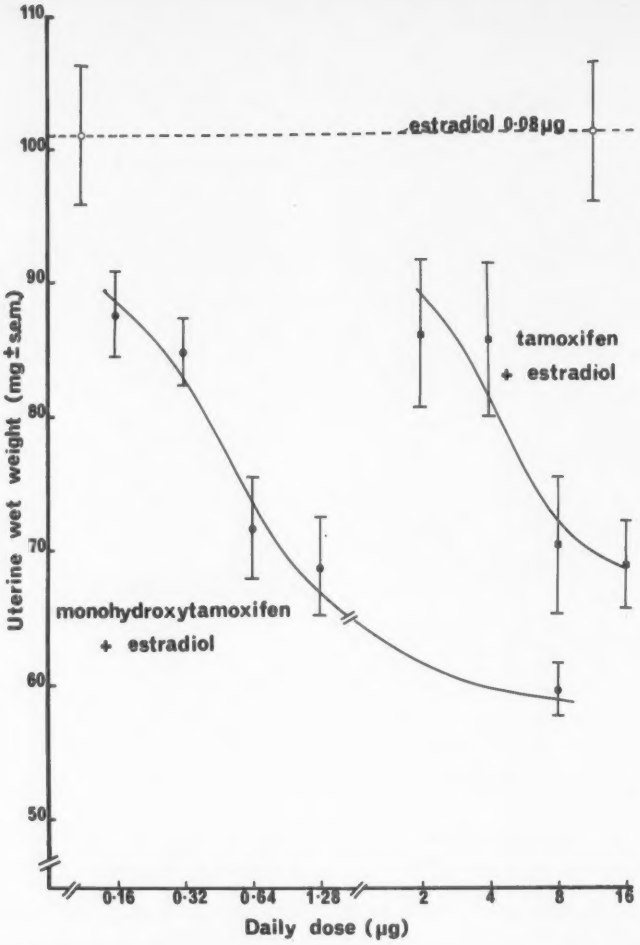


FIGURE 2.—Antiestrogenic effect of monohydroxytamoxifen and tamoxifen administered simultaneously (sc) with estradiol (0.08 µg/day) in the 3-day, immature rat uterine weight test. All injections were given in 0.1 ml of peanut oil, with multiple injections at separate sites. Animals were killed on the 4th day and the uteri were weighed wet on a torsion balance. Results = mean ± SEM with 10 rats in each group (data *from* ref 12).

ative of tamoxifen has a much-reduced binding affinity (Allen et al, unpublished findings).

As yet, there are no data on the binding of [$^3$H] monohydroxytamoxifen to the estrogen receptor, but several studies have shown that [$^3$H] tamoxifen binds directly to the cytoplasmic estrogen receptor from rat uteri (15,25,26) and rat mammary tumors (27).

Just as with other antiestrogens (28–30), the administration of tamoxifen (31,32) and monohydroxytamoxifen (33) to rats results in the translocation of cytoplasmic estrogen receptors to the nuclear compartment. Probably as a result of this event, cytoplasmic progesterone receptor synthesis is initiated (19,34). A time-course experiment, in which immature rats were injected with tamoxifen (25 µg) every 12 hours to maintain high levels of ligand in the blood, is illustrated in figure 6. Unlike the antiestrogen, nafoxidine (which maintains high levels of nuclear estrogen receptors for prolonged periods [28]), the nuclear pool of "tamoxifen" estrogen receptor complexes slowly decreases with

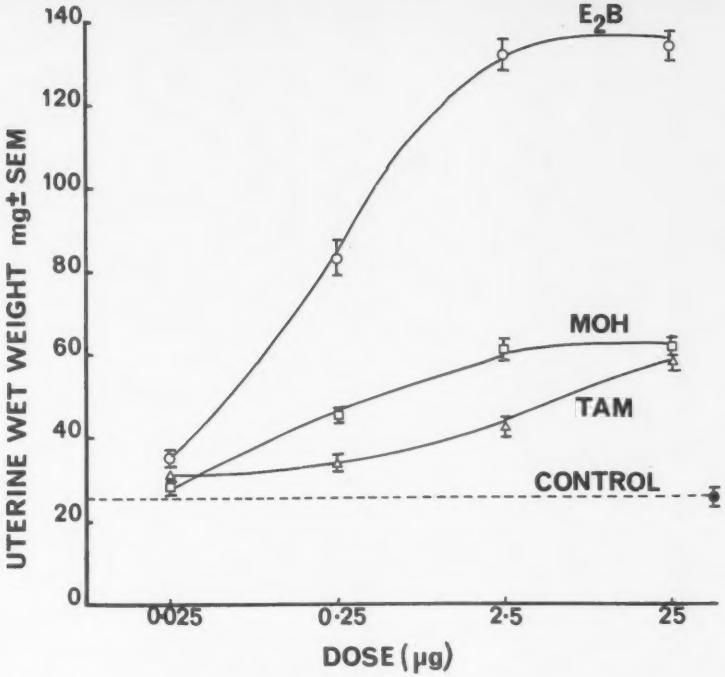


FIGURE 3.—Effect of various doses of estradiol benzoate ($E_2B$), tamoxifen (TAM), or monohydroxytamoxifen (MOH) on immature rat uterine weight, as determined 48 hrs after sc administration in 0.1 ml of peanut oil; 5 rats/group (data *from* ref 19).

time as the concentration of cytoplasmic progesterone receptor complexes increases. This series of biochemical events has been referred to as "processing" (35), and it has been suggested that antiestrogens are less readily able to undergo processing than are estrogens (36).

The situation with tamoxifen in vivo is complicated by the conversion to monohydroxytamoxifen. Figure 7 shows an experiment illustrating that nuclear monohydroxytamoxifen-estrogen receptor complexes are "processed" as readily as estradiol-estrogen receptor complexes, although total uterine DNA levels do not increase with monohydroxytamoxifen. If there are significant levels of monohydroxytamoxifen-estrogen receptor complexes present in the nucleus during tamoxifen therapy, and if these are processed, these conditions may account for the decrease and apparent processing of nuclear tamoxifen-estrogen receptor complexes (fig 6).

## ANTITUMOR PROPERTIES OF TAMOXIFEN

The rat mammary carcinoma model induced by DMBA (39) has received the most attention as a hormone-dependent system. The tumors are believed to be primarily dependent on prolactin (40,41), and it has also been suggested that estrogen has a direct role (42). The presence of both prolactin (43) and estrogen receptors (44) in the tumors indicates a potential role for both hormones.

With the interest in the direct estrogen receptor-mediated control of human breast cancer growth, the initial interest in the mechanism of action of antiestrogens in DMBA-induced rat mammary tumors centered on the ability of antiestrogens to block the direct effect of estrogen in the tumor. Terenius (45) suggested that antiestrogens produce their antitumor effects by receptor blockade; his observations were later confirmed (46–49). DMBA-induced tumors with low levels of estrogen receptors are less responsive than tumors with high levels of estrogen receptors (48). Similarly, transplantable rat mammary tumors LMCI (with very low levels of estrogen receptors) do not respond to tamoxifen therapy (50). Many other factors may be involved in this apparently straightforward conclusion, and the theory must be re-examined.

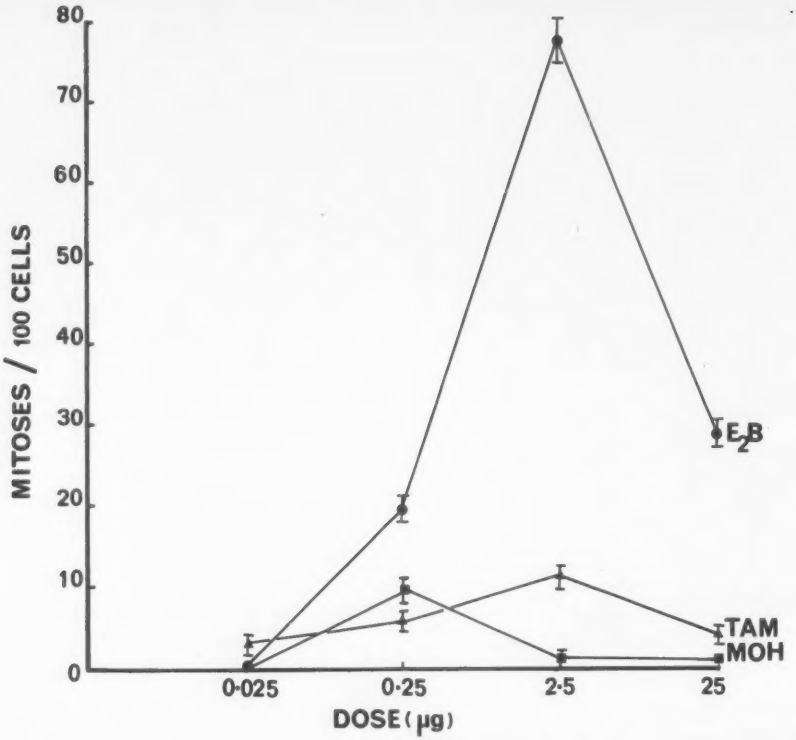


FIGURE 4.—Effect of various doses of estradiol benzoate ($E_2B$), tamoxifen (TAM), or monohydroxytamoxifen (MOH), on % mitosis in uterine luminal epithelial cells, 48 hrs after sc administration in 0.1 ml of peanut oil. Animals were injected with 100 μg of colchicine in 0.1 ml of saline, 7 hrs before being killed; uteri were fixed in 4% formal saline solution and embedded in paraffin. Sections (5-μm) were taken from the midportion of uterine horns and stained with hematoxylin and eosin. 6 random fields/uterus were examined with 5 immature rats/group (n = 30) (data *from* ref 19).

Young DMBA-induced rat mammary carcinomata readily regress in response to ovariectomy; older carcinomata become increasingly refractory (51). We have found that the majority of young DMBA-induced tumors (< 150 days after DMBA) regress in response to 3 weeks of tamoxifen (50 μg/day) or 2-bromoergocryptine (CB154: an inhibitor of prolactin release, 1.5 mg/day) therapy; older tumors are more resistant to these antihormone therapies (table 1).

To investigate these findings, we measured estrogen and progesterone receptor concentrations in young and old DMBA-induced tumors. Although estrogen receptor concentrations decrease somewhat with age, the progesterone receptor concentrations are much more sensitive and decrease dramatically (fig 8). Thus, the hormone sensitivity of the tumor decreases with the age of the tumor and this is associated with a progressive loss of the estrogen and progesterone receptor systems. These observations suggest why antihormone therapy is ineffective, rather than how tamoxifen produces its effects. The blockage of estrogen receptors by tamoxifen is a reproducible effect, but the ability to reverse tamoxifen-induced regression by increasing prolactin output with perphenazine (54) suggests that the tumor estrogen receptor blockade does not produce an irreversible insult in the tumor cell.

Figure 9 summarizes the endocrine effects of tamoxifen in the rat. Tamoxifen can inhibit estrogen synthesis in the rat either directly (55) or possibly by disrupting the correct sequence of gonadotropin secretion (56). We have recently observed that tamoxifen is sufficiently estrogenic after long-term

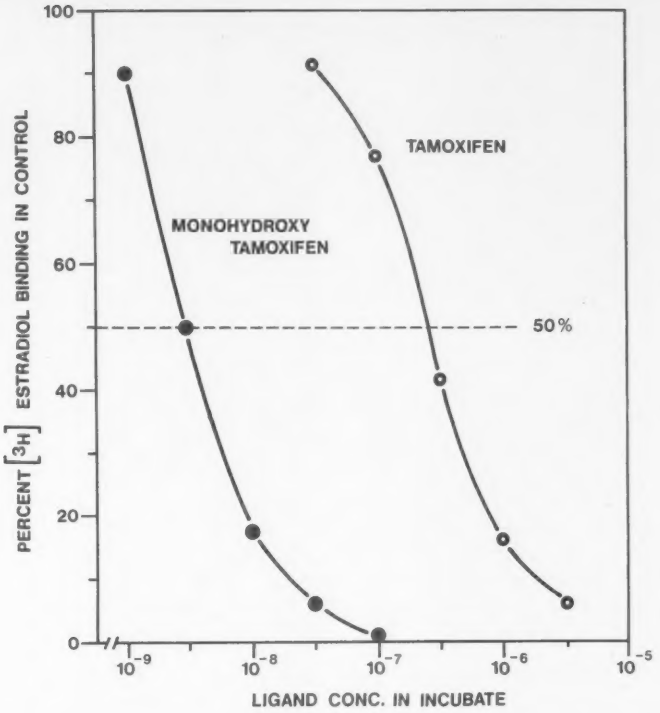


FIGURE 5.—Inhibition of [$^3$H] estradiol binding (incubation concentration $5 \times 10^{-9}$ mols/liter) to rat uterine estrogen receptors, by increasing concentrations of monohydroxytamoxifen or tamoxifen. Incubations were undertaken for 4 hrs at 0°C–4°C, and free radioactive ligand was removed by incubations with dextran-coated charcoal (7). Tamoxifen produced 50% inhibition of [$^3$H] estradiol binding at $2.35 \times 10^{-7}$ mols/liter; monohydroxytamoxifen produced a 50% inhibition at $3.25 \times 10^{-9}$ mols/liter.

administration to reduce ovariectomized rat luteinizing hormone (LH) levels (Jordan et al, unpublished observations). Clearly, a badly timed and inadequate production of estrogen will inhibit the cyclic release of prolactin. Antiestrogens can inhibit estrogen-stimulated elevations in circulating prolactin (57); intact rats treated with tamoxifen for a prolonged period have circulating prolactin levels that are not statistically different from animals previously ovariectomized (Jordan et al, unpublished observations).

We suggest that tamoxifen produces a multitude of endocrine effects that affect tumor homeostasis, the net result of which is tumor regression. Whether tamoxifen is actually cytotoxic is debatable, but the inability of the tumor cells to enter the cell cycle and be replaced must result in overall cell loss. However, cell selection pressure and the development of tamoxifen-resistant cells will eventually result in tumor regrowth.

## Laboratory Model for Adjuvant Antihormone Therapy

Based on Emmens' (9) observations that the sc administration of 5 mg of tamoxifen for 2 consecutive days produces a long-term antiestrogenic effect in rats, we found that administering this dosage to 50-day-old rats at the same time as DMBA reduces the number of mammary carcinomata induced (58). However, this is a poor model for adjuvant therapy, where the aim is to destroy micrometastases. Carcinogenesis with DMBA is dependent on the age of the rats (39) and the correct hormonal environment (59). At the subcellular level, prolactin-stimulated thymidine incorporation in breast tissue has been correlated with DMBA-induced car-

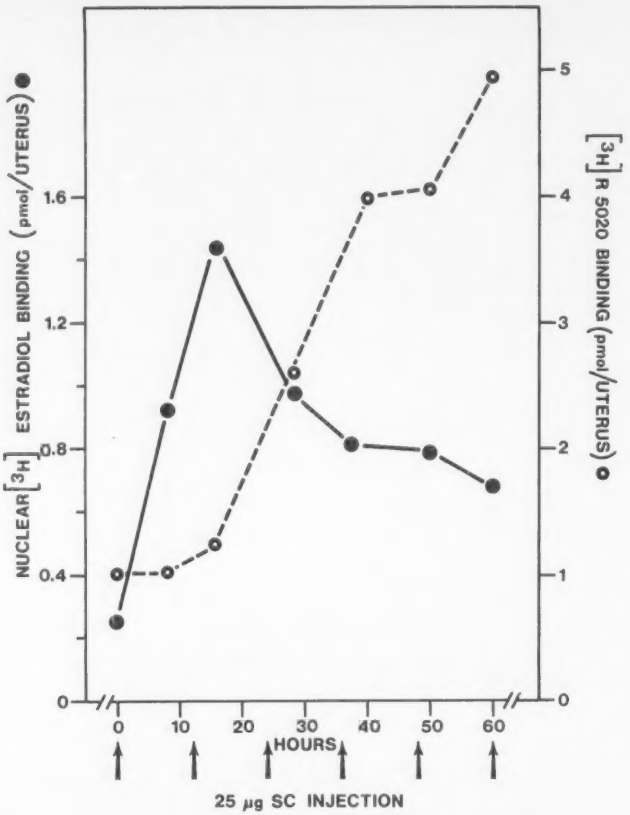


FIGURE 6.—Effect of 12 hourly sc injections (25 μg) of tamoxifen on immature rat uteri nuclear estrogen receptor levels (●) or uterine cytoplasmic progesterone receptor levels (○). Nuclear estrogen receptor levels were determined using the method described by Katzenellenbogen (38) and progesterone receptor levels were determined using [$^3$H]R5020 by the method described by Vu Hai and Milgrom (37). Determinations were made on paired uteri with 8 immature rats in each group.

cinogenesis (60), which suggests that circulating levels of both prolactin and breast tissue-DNA synthesis are essential for the early stages of any neoplastic changes.

Tamoxifen will reduce estrogen-stimulated elevations in circulating prolactin (57) and inhibit DNA synthesis in breast cancer cells in tissue culture (15) and rat uteri (19), so the simultaneous administration of DMBA and tamoxifen may inhibit carcinogenesis, rather than destroy malignant cells. Another factor is the potential requirement of metabolic activation of the carcinogen prior to producing malignant transformation (61). There is no information on whether tamoxifen will alter the liver or breast metabolism of DMBA. After considering all these factors, we decided to delay therapy for 4 weeks after DMBA when it was assumed that malignant transformation had taken place and microfoci of damaged cells would be present. This situation might be analogous to the situation in which micrometastases are present in a patient after the primary tumor has been surgically removed.

In the first experiment (fig 10), the administration of different daily doses of tamoxifen (0.2, 3, 50, or 800 μg daily) produced a dose-related inhibition of tumor appearance. However, the percentage of rats with tumors within groups increased with time so that, by 200 days after DMBA administration, animals had tumors, and tumors that arose after tamoxifen therapy were nearly all responsive to a second antihormonal modality, ovariectomy (62).

Since tamoxifen administered for a short period did not completely cure animals, we compared the

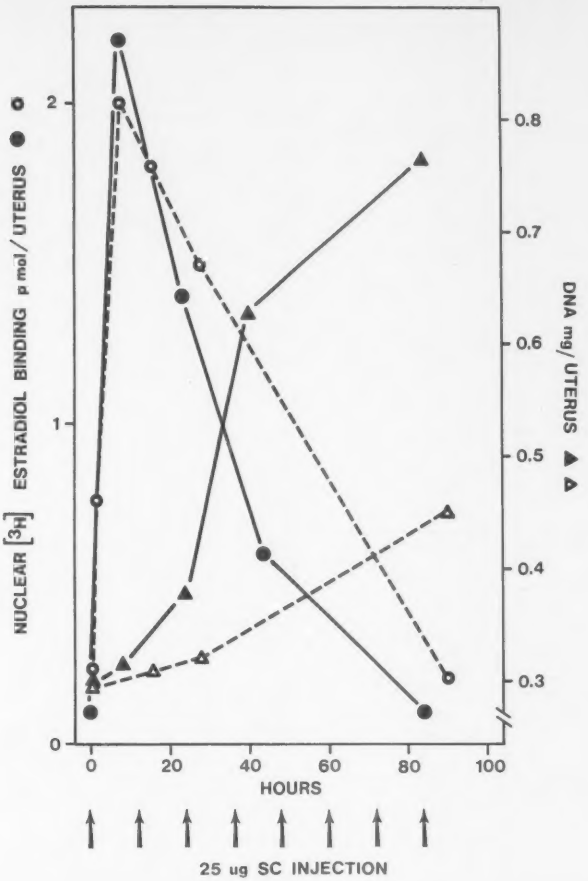


FIGURE 7.—Effect of 12 hourly sc injections (25μg) of estradiol benzoate (solid lines) or monohydroxytamoxifen (broken lines) on immature rat uterine nuclear estrogen receptor levels (●○) or whole uterine DNA levels (▲△). Nuclear estrogen receptor levels were determined using the method described by Katzenellenbogen (38). Determinations were made on paired uteri with 8 immature rats in each group.

effects of tamoxifen with those of a more potent antiestrogen—monohydroxytamoxifen. Although 50 μg of monohydroxytamoxifen daily is more effective at reducing ovariectomized rat estrogen receptor concentrations than 50 μg of tamoxifen daily (62), monohydroxytamoxifen appears to be a much weaker antitumor agent (fig 11).

Overall, these results are in marked contrast to the reports that MCF 7 human breast cancer cells in tissue culture are destroyed by high concentrations of antiestrogens (15). Thus, we decided to determine whether the initiation of 4 weeks of therapy as early as 5 days after DMBA would produce a greater proportion of animals in the tumor-free category. Under these conditions, assuming a minimum cell cycle time of 20 hours for a DMBA-induced tumor cell (63) and a *single* malignant transformed cell with no cell death, there would be, at the most, microfoci of 32 malignant cells. Tamoxifen (50 μg daily) (64) and monohydroxytamoxifen (100 μg daily) (unpublished observation) were unable to completely cure animals.

The length of action of tamoxifen is directly related to the dose initially administered. Thus, the dose-related delay in tumor appearance suggests that the malignant cells are not all destroyed, even though their growth and replication is inhibited in the continued presence of the drug. The same ar-

TABLE 1.—Mean DMBA-induced rat mammary tumor area before and after 3 wks of therapy with 50 μg of tamoxifen/day (sc in 0.1 ml of peanut oil) or 1.5 mg of CB154 [bromoergocryptine]/day (sc in 0.2 ml 20% DMSO)*

| Group | Treatment | No. of tumors | Tumor area | |
|---|---|---|---|---|
| | | | Before therapy (± SEM)† | After therapy (± SEM)† |
| A | Control | 23 | 3.82±0.4 | 4.14±0.51 |
| | Tamoxifen | 17 | 4.58±0.55 | 2.31±0.42‡ |
| | CB154 | 8 | 4.14±0.79 | 1.36±0.44‡ |
| B | Control | 26 | 4.78±0.62 | 6.99±1.07 |
| | Tamoxifen | 28 | 4.62±0.36 | 4.4 ±0.78 |
| | CB154 | 8 | 4.36±0.77 | 7.83±3.18 |

*Controls were injected with peanut oil. Tumor area was determined with calipers and calculated using the formula $\Pi \times \frac{\text{length}}{2} \times \frac{\text{width}}{2}$. Group A animals were between 100 and 150 days after DMBA and Group B animals were between 200 and 250 days after DMBA.

†$P$ values were determined by comparing means of treatment before or after therapy.

‡$P < 0.01$, Student's t test.

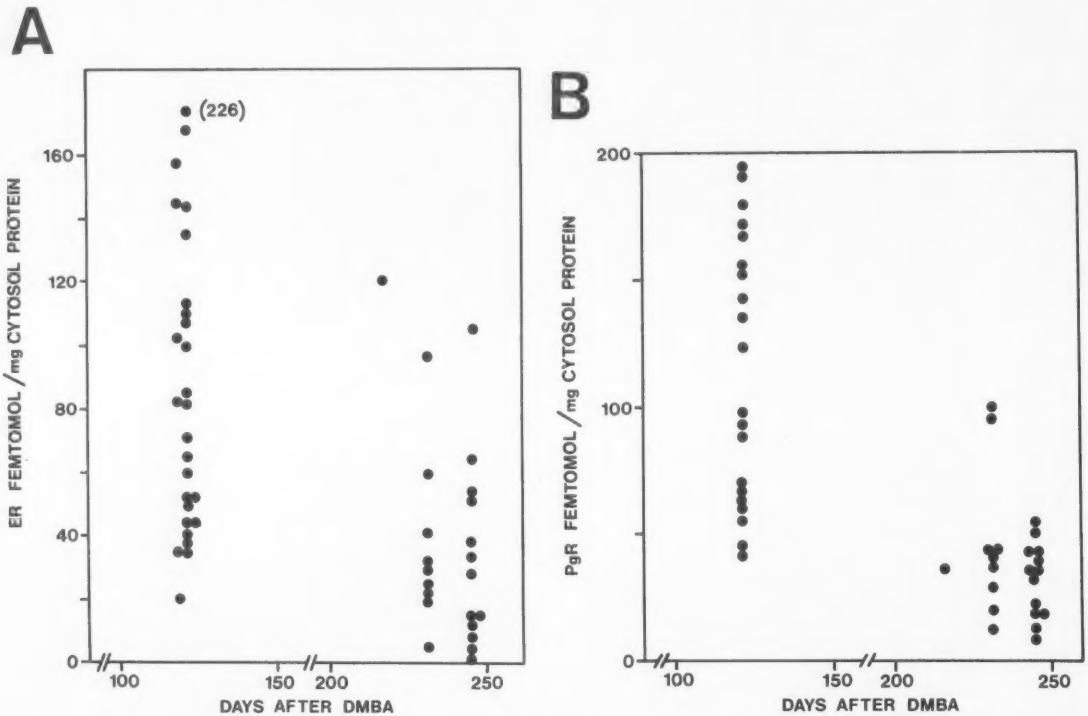


FIGURE 8.—Effect of DMBA-induced rat mammary tumor age on the concentration of (A) cytoplasmic estrogen receptor (ER) concentration and (B) cytoplasmic progesterone receptor (PgR) concentrations. Tumor biopsies were excised from anesthetized rats and the tissue was immediately frozen in liquid $N_2$. Homogenates were prepared in 2 ml Tris buffer (tris 0.01 mol/liter, EDTA 0.0015 mol/liter, dithiothreitol 0.005 mol/liter, pH 7.4), and 100,000 × $g$ supernatants (cytosols) were prepared. Estrogen and progesterone receptor levels were determined by the methods described in refs 53 and 31, respectively. Cytosol protein was measured by the method of Lowry et al (53). All specimens of tumor tissue were taken when the rat was at diestrus.

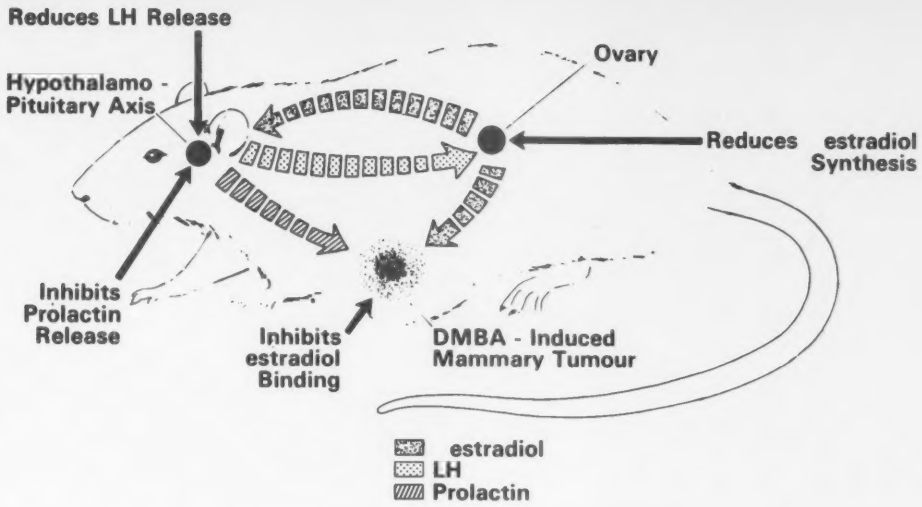


FIGURE 9.—Endocrine effects of tamoxifen in rats with DMBA-induced rat mammary carcinomata.

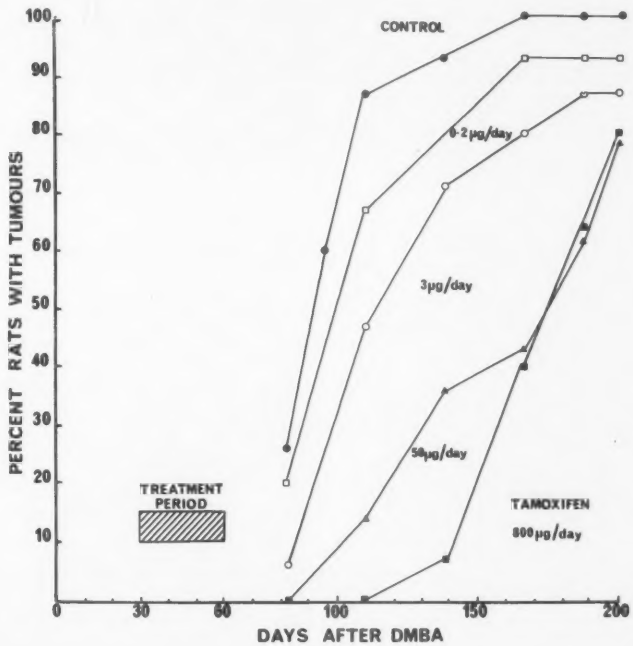


FIGURE 10.—Effect of sc administrations of different daily doses of tamoxifen (0.2, 3, 50, or 800 µg/day in 0.1 ml of peanut oil, 5 days/wk) between 30 and 60 days after DMBA on the % of rats (15/group) developing tumors up to 200 days after DMBA administration (20 mg DMBA in 2 ml of peanut oil). Animals in the control group were injected with peanut oil between 30 and 60 days after DMBA.

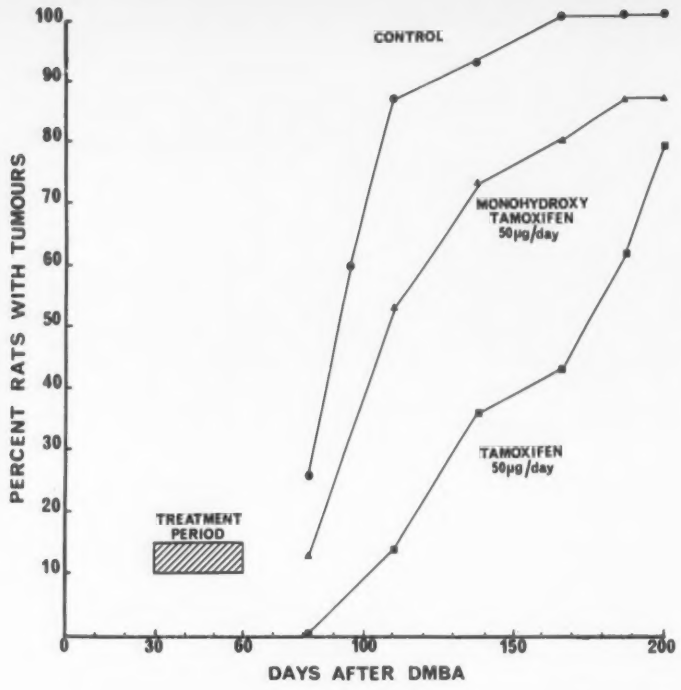


FIGURE 11.—Effect of daily sc administrations (5 days/wk) of either monohydroxytamoxifen (50 μg in 0.1 ml of peanut oil) or tamoxifen (50 μg in 0.1 ml of peanut oil) between 30 and 60 days after DMBA on the % of rats (15/group) developing tumors up to 200 days after DMBA. Controls were injected with peanut oil.

gument applies to monohydroxytamoxifen. When the drug has been cleared from the rat and the normal cyclic hormonal environment returns, the malignant cells are reactivated and develop into palpable tumors.

This suggestion was found valid when a short course of tamoxifen therapy (50 μg daily for 4 weeks) was compared with continuous therapy (50 μg daily for 24 weeks) (fig 12). A short course of therapy reduced tumor numbers (28 tumors in therapy vs. 44 in controls 200 days after DMBA). Continuous therapy kept the majority of rats tumor-free (two tumors present within the group 200 days after DMBA).

In experiments with continously administered monohydroxytamoxifen, a similar effect was observed (62). Again, tamoxifen appeared to be more potent. In conclusion, it is clearly advantageous to maintain blood levels of antiestrogens for prolonged periods to effectively suppress tumor production in this model.

## CLINICAL RELEVANCE

The present laboratory findings have three major areas of clinical application. First, the antiestrogen-stimulated increases in rat urine progesterone receptors may have important therapeutic implications for the future clinical management of endometrial carcinoma and the resulting metastases. Tamoxifen inhibits the binding of estradiol to estrogen receptors derived from human uterine adenocarcinomata (65); therefore antiestrogens might inhibit estrogen-stimulated cell division and proliferation.

Furthermore, a recent report (66) has shown that a short course of tamoxifen therapy will increase the endometrial content of progesterone receptors. In the laboratory, a combination of nonsteroidal antiestrogens and progestins has a synergistic effect on the growth inhibition of rat uterine adenocarcinoma cells in tissue culture (67). If tamoxifen could also sensitize the human carcinomata to the

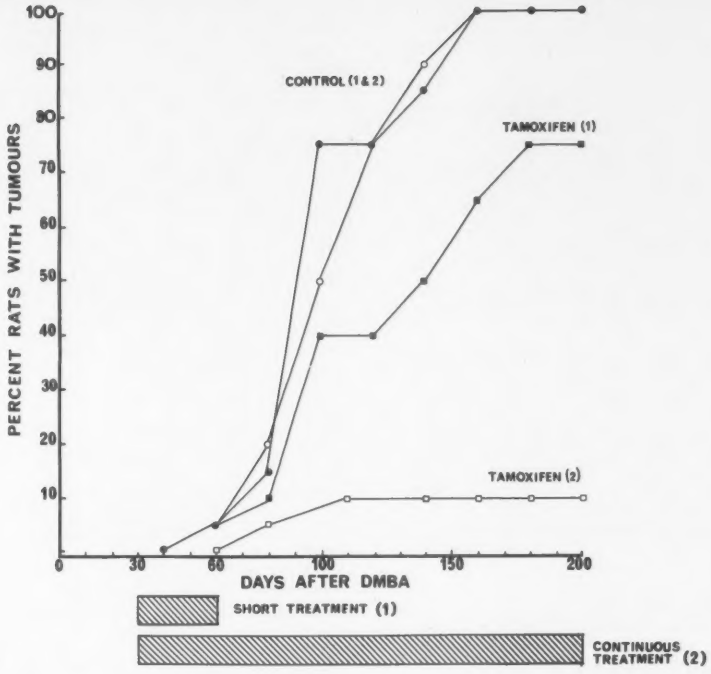


FIGURE 12.—Effects of daily sc administration (5 days/wk) of tamoxifen (50 μg in 0.1 ml of peanut oil) for a short treatment period (30–60 days after DMBA) or continuous treatment period (30–200 days after DMBA) on the % of rats (20/group) developing tumors. The experiments were conducted at different times and the related controls (1 and 2) received peanut oil alone.

pharmacologic effects of progestin therapy in the clinic, then a study of all practical therapeutic regimens should be undertaken.

Second, the finding that estrogen receptor-poor transplantable LMCI tumors do not respond to tamoxifen therapy, but that young, estrogen receptor-rich, DMBA-induced rat mammary carcinomata do respond, underlines the clinical findings that advanced breast cancers that are estrogen receptor-rich respond more readily to tamoxifen therapy than do estrogen receptor-poor tumors (4). Although the case for using estrogen receptor assays as a predictive test for ablative surgery is well-defined (only one third of the patients will respond to treatment), the case for using hormone receptor assays prior to starting tamoxifen therapy (which has few toxic side effects) is less clear.

The low reported incidence of side effects is fundamental to the third clinical application of tamoxifen. The estrogen receptor status of the primary tumor is a useful prognostic factor for predicting whether a breast tumor will recur early or late (68,69). Primary tumors that are estrogen receptor-poor recur earlier than do estrogen receptor-rich tumors. Many large international studies are using tamoxifen as an adjuvant to surgery in breast cancer, but it is not established whether estrogen receptor-rich tumors will be controlled more effectively than estrogen receptor-poor tumors. If estrogen receptor-rich tumors respond to tamoxifen, then the drug may be able to control tumor recurrence in a highly selected subpopulation of patients.

The question arises as to how long therapy should be continued. Although there are many fundamental biologic differences between the DMBA-induced rat mammary carcinoma model and human breast cancer, the principles for the control of hormone-dependent growth may be similar. As shown in the present experiments, a short course of tamoxifen therapy (1 month in a rat lifetime of 3 years) does not destroy all the hormone-dependent tumor cells, and only longer treatment cycles are effective. Determining whether human breast cancer cells are

protected from antiestrogen action in vivo must await the completion of the clinical trials of 1- and 2-year treatment regimens.

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# Studies on the Metabolism and Pharmacokinetics of Tamoxifen in Normal Volunteers

**Hugh K. Adam,* John S. Patterson, and John V. Kemp[1,2]**

## SUMMARY

**The densitometric analytic procedure used for tamoxifen can also be used to quantify its desmethyl metabolite. In a study involving six healthy male volunteers, tamoxifen tablets were shown to be as bioavailable as a solution of tamoxifen citrate. After administration of a single dose of 20 mg, peak serum levels of tamoxifen were 42 ng/ml; those of the metabolite were 12 ng/ml. The half-lives of the drug and metabolite were approximately 4 and 9 days, respectively, after a single dose. After three widely separated single doses, a reversible increase in elimination half-life occurred.**

[Cancer Treat Rep 64:761–764, 1980]

Since its clinical introduction in the early 1970s tamoxifen (Nolvadex, the property of Imperial Chemical Industries, Ltd) has been prescribed with increasing frequency as a palliative treatment for advanced breast cancer. Until recently, knowledge of its pharmacokinetics and metabolism in humans was limited to that obtained by Fromson et al (1) after administration of single oral doses of $^{14}$C-tamoxifen to female patients. No methodology was available to analyze the drug or its metabolites in biologic samples.

Three analytic methods have recently been described to quantify circulating tamoxifen and its metabolites. A procedure based on high-pressure liquid chromatography, after conversion to a phenanthrene derivative, has been reported by Mendenhall et al (2). The second procedure, involving gas chromatography coupled to a high-resolution mass spectrometry, was described by Gaskell et al (3). Both of these methods have been reported to allow the measurement of both tamoxifen and a hydroxylated metabolite in biologic samples. The method of Adam et al (4) is based on thin-layer chromatography with photochemical conversion in situ to a fluorescent phenanthrene derivative, followed by densitometric analysis of the thin-layer chromatogram.

This paper describes the use of this last method to investigate the metabolism and pharmacokinetics of tamoxifen in healthy male volunteers and compares the results with those previously obtained in female patients.

## MATERIALS AND METHODS

Six healthy male volunteers (mean age, 33.4 years; mean weight, 75.3 kg) gave their informed consent and participated in the study. Each subject was given a single oral dose of 20 mg of tamoxifen on each of three occasions, separated by at least 28 days. The study was of open cross-over design such that two subjects received each formulation at the three time points. The three phases were designated periods 1, 2, and 3 and began on Days 1, 42, and 70, respectively.

The following formulations of tamoxifen were used:

*a*. Two 10-mg commercially available tablets (Nolvadex) and 600 ml of water;

*b*. One 20-mg experimental tablet formulation and 600 ml of water; and

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[2]We wish to acknowledge the invaluable assistance of Sisters J. Young and A. Shields and to thank Dr. R. Ferguson for statistical assistance and advice.

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c. An aqueous solution of tamoxifen citrate, 30.3 mg (containing 20 mg of tamoxifen) in 600 ml of water.

Two subjects took an additional 20 mg of tamoxifen (formulation c) on Day 215, ie, 145 days after period 3.

Blood samples were obtained by venipuncture immediately before the subjects received their doses, after 1, 3, 5, 6, 12, and 24 hours, and on Days 2, 8, 16, and 20 after receiving each dose. Serum was harvested within 4 hours of obtaining the sample and stored at –20°C until analyzed by the procedure of Adam et al (4), with simultaneous determination of tamoxifen and its *N*-desmethyl metabolite (5).

Statistical evaluation was by analysis of variance (ANOVA).

## RESULTS

Figure 1 shows a typical calibration curve derived from simultaneous analysis of samples containing both tamoxifen and its *N*-desmethyl metabolite with reference to the internal standard previously described (4). The lower limit of reliable quantification from 1 ml of serum was 2.5 ng/ml for both compounds. Examination of sera from this study failed to detect any of the metabolites found in excreta by Fromson et al (1).

Estimation of tamoxifen levels showed that maximal levels of 35 to 45 ng/ml were achieved about 5 hours after administration of either tablet formulation or the solution. It was also possible to calculate the terminal half-life and area under the curve ($AUC_\infty$) for tamoxifen. ANOVA on the log-transformed data showed that the mean tablet $AUC_\infty$ (2606 ng/ml/hour) was not significantly different from that of the reference solution, mean value (2295 ng/ml/hour). Equally, the mean terminal half-lives were not significantly different (solution, 101 hours; tablets, 111 hours). For the comparison the 95% confidence limits, expressing the tablet result as a percentage of the reference solution, were from 87% to 148%. This result shows that the commercially available tablet formulation is at least as bioavailable as the reference solution.

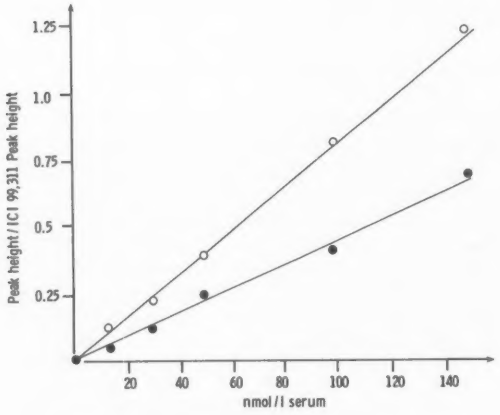


FIGURE 1.—Calibration series of tamoxifen (○) and desmethyltamoxifen (●) obtained by simultaneous estimation.

However, ANOVA showed that the pharmacokinetic parameters were significantly different between the different periods of the study ($P < 0.01$). The mean half-life for period 1 was 91.2 hours, that for period 2 was 98.4 hours, and for period 3 the value was 156 hours.

These differences in half-lives were reflected by a corresponding increase in $AUC_\infty$ as follows: period 1, 1844 ng/ml/hour; period 2, 3092 ng/ml/hour; and period 3, 3479 ng/ml/hour. Figure 2 illustrates the differences between the mean tamoxifen concentrations of periods 1 and 3 in the study.

Although the desmethyl metabolite could be detected in all periods of the study, insufficient data were available to calculate the pharmacokinetic parameters for this compound. Peak concentrations ranged from 10 to 20 ng/ml, and elimination was slow; detectable levels were still present 288 hours after administration.

Part 2 of the study (Day 215) in two subjects produced the results shown in figure 3. The elimination half-lives of tamoxifen were 72 and 84 hours, respectively. Levels of metabolite built up slowly, to a peak of about 10 ng/ml. Elimination was slow, with terminal half-lives of 9.8 and 9.2 days, estimated from data obtained at times $\geq 8$ days.

## DISCUSSION

Fromson (1), using $^{14}C$-tamoxifen in female patients, showed that peak levels of total radioactivity corresponding to 100 ng/ml were achieved after a single 20-mg dose. About 25% of this radioactivity corresponded to unchanged tamoxifen. These workers also showed that decay was slow, with detectable radioactivity still present 14 days after administration.

In female patients, it has been reported (6) that peak levels of tamoxifen of 15 to 25 ng/ml were obtained after a single 10-mg dose and that a single dose of approximately 10 mg/m$^2$ yielded a median concentration of 16 ng/ml (7). In both cases, elimination was slow.

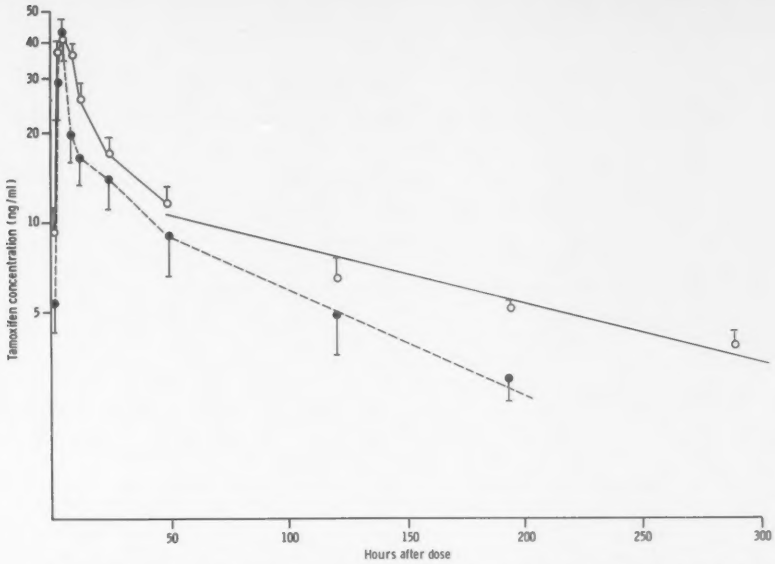


FIGURE 2.—Mean tamoxifen concentrations/± SE in period 1 (●) and period 3 (○) of study.

The peak tamoxifen levels achieved in the present study (35 to 45 ng/ml from a 20-mg dose) and the long terminal half-life agree with the above findings and suggest that no major pharmacokinetic difference exists in the handling of taxomifen between healthy male subjects and female patients with breast cancer. The studies also show that the kinetics are linear over this dose range.

The finding that the elimination half-life of tamoxifen increased with successive doses is interesting; it may be due to a reversible and limited inhibition of the hepatic metabolism of the drug, although the mechanism is unclear. The fact that the terminal half-life in two subjects returned to initial values after a 145-day interval between doses supports this hypothesis. The inhibitory effect appears to be self-limiting; Patterson et al (8) and Fabian and Sternson (7) showed that steady-state levels of tamoxifen are achieved in patients after 4 weeks of multiple-dose therapy and then remain constant for periods of up to 26 weeks. The mean half-life in period 3 of the present study (156 hours) fits with this accumulation pattern and suggests that the effect is maximal at this point.

The metabolites identified by Fromson et al (1) were obtained after hydrolysis of fecal extracts. All contain hydroxyl groups; these metabolites are probably excreted as conjugates. We failed to find any of these metabolites in human serum. Daniel et al (9) found low levels of 4-hydroxytamoxifen in human serum after repeated drug administration. These workers also reported the presence, again at low concentrations, of a metabolite that appears to be isomeric with the 4-hydroxy metabolite. Mendenhall et al (2) reported levels of an unidentified metabolite.

It is suggested that the hydroxylated metabolites, once formed, are rapidly conjugated and eliminated via the bile, which may explain the low or nondetectable levels of these metabolites in serum. The parent drug and its desmethyl metabolite cannot be readily conjugated, and these compounds must be further metabolized prior to their excretion.

Workers in our laboratories have shown that the *N*-desmethyl metabolite has antiestrogenic properties in animals virtually identical to the characteristics exhibited by tamoxifen. Hence, any consideration of the relationship between the pharmacokinetics of tamoxifen and its clinical efficacy should allow for the possible supportive role of the metabolite. Furthermore, any chemical measurements, such as estrogen receptor determinations after withdrawal of tamoxifen therapy, should allow adequate time for the elimination of both the drug and this metabolite.

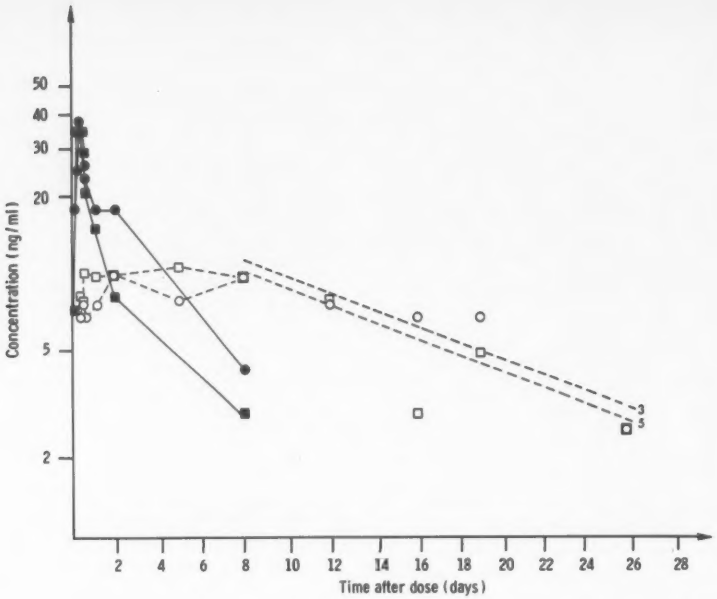


FIGURE 3.—Levels of tamoxifen (solid symbols) and desmethyltamoxifen (open symbols) in two subjects given 20 mg of tamoxifen in solution 145 days after period 3 (● and ○ volunteer 3; ■ and □ volunteer 5).

At present, insufficient data are available to establish the relevance of the pharmacokinetic findings with respect to the clinical response to tamoxifen therapy. One attempt to do this, in a study involving 43 postmenopausal patients with advanced breast cancer (8), failed to establish a correlation between response and steady-state serum levels of tamoxifen or desmethyltamoxifen. Further studies are in progress to clarify this aspect.

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# Clinical Pharmacology of Tamoxifen in Patients with Breast Cancer: Comparison of Traditional and Loading Dose Schedules[1]

**Carol Fabian,* Larry Sternson, and Marge Barnett[2]**

## SUMMARY

**Tamoxifen blood levels were measured in patients with metastatic breast cancer after single-dose and continuous administration, using a traditional dose schedule of 10 mg/m² twice daily; a single daily dose schedule of 20 mg/m²; and several loading dose schedules of 20–80 mg/m² twice daily × 7 days, then a 20-mg/m² single daily dose thereafter. Using the traditional 10-mg/m² twice daily schedule, values after continuous administration at the time clinical responses were observed were ≥ 10× peak values observed after a single dose. Using a loading dose schedule of ≥ 40 mg/m² twice daily × 7 days, values known to be associated with a response were present by 3 hours, as opposed to ≥ 7 days with the traditional schedule. Levels obtained at peak and trough times with the once-daily schedule suggest tamoxifen may safely be given on a once-daily basis, particularly after the first 8 weeks of therapy. Half-life of tamoxifen after continuous administration is prolonged, and levels obtained for up to 6 weeks after drug discontinuation suggest that false-negative cytoplasmic estrogen receptor (ER) determinations may be obtained if tissue for ER is sampled within 4–6 weeks of prolonged tamoxifen administration.**

[Cancer Treat Rep 64:765–773, 1980]

Pharmacokinetic studies of tamoxifen and its major metabolites have several potential clinical applications: (*a*) delineation of a drug concentration consistent with clinical response; (*b*) determination of an appropriate dose schedule to rapidly achieve drug levels consistent with response; (*c*) delineation of an appropriate time interval after discontinuing tamoxifen to sample tissue for cytoplasmic estrogen receptor (ER) so that false-negative values are not obtained; and (*d*) aid in design of combined chemotherapy-hormone studies. With these goals in mind, we measured levels of tamoxifen and desmethyltamoxifen in patients with metastatic breast cancer after a single dose and after continuous administration of tamoxifen. We used a traditional dose schedule of 10 mg/m² twice daily; a single daily dose schedule of 20 mg/m², and several loading dose schedules.

Fromson et al (1) performed the early pharmacokinetic studies utilizing $^{14}C$-labeled tamoxifen. Feces and urine were collected in two patients after they received a single dose, equivalent to 12 mg/m². Maximum tamoxifen levels were reached 4–7 hours after administration. An initial half-life of 7–14 hours was observed, with a secondary half-life of 7+ days. Prolonged fecal excretion over several weeks was noted. Sampling times in our initial patients were based on this early work.

## MATERIALS AND METHODS

A total of 64 patients with metastatic breast cancer have been entered in the study, which is ongoing. Patients may have had prior treatment with

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chemotherapy or hormone therapy other than tamoxifen. Evaluable, but not necessarily measurable, disease was required for patient entry; therefore, a response determination could not be made in all patients. All patients had the following tests performed before treatment: cbc and platelet count; blood calcium and estradiol levels; liver and renal functions; roentgenograms of spine, pelvis, skull, and chest; and a liver scan. ER analyses were performed where possible by the dextran-coated charcoal technique (2). Specimens containing < 3 fmols of receptor/mg of cytosol protein were considered negative; 3–9 were considered borderline; and those containing ≥ 10 were considered positive. Clinical examination, roentgenograms of measurable lesions, cbc, platelet counts, liver function, and calcium measurements were repeated every 4–8 weeks.

Samples for tamoxifen assay were drawn in heparinized tubes and frozen until analysis. The assay utilized direct extraction, photochemical activation, and chromatographic analysis, as previously reported (3,4). Results are reported as nanograms of drug or metabolite per milliliter of whole blood. One nanogram of tamoxifen per milliliter is equal to $2.7 \times 10^{-9}$ mols/liter.

Twenty-three patients received a tamoxifen dose of 10 mg/m² twice daily. Blood levels were sampled 0, ½, 3, 6, 9, and 12 hours after administration, and then 3 and 12 hours after administration every 4 weeks. Nine patients received a tamoxifen dose of 10 mg/m² twice daily, but their blood samples were drawn 3 and 12 hours after administration on Days 1, 4, and 7; weekly thereafter for a total of 8 weeks; and then 3 and 12 hours after administration every 8 weeks.

Thirteen patients received a single daily dose of 20 mg/m² of tamoxifen. Their blood samples were drawn 3 and 24 hours after administration on Days 1, 4, and 7; weekly for 8 weeks; and then every 8 weeks. Twenty patients received a loading dose for 1 week, then a single dose of 20 mg/m² daily thereafter. The loading dose was 20 mg/m², twice daily, for seven patients; 30 mg/m², twice daily, for four patients; 40 mg/m², twice daily, for seven patients; and 80 mg/m², for two patients. Blood sampling in all patients receiving the loading dose was performed 3 and 12 hours after administration of the drug on Days 1, 4, and 7; 3 and 24 hours after administration weekly during Weeks 2–8; and 3 and 24 hours after administration every 8 weeks thereafter.

Samples were also obtained after patients had failed treatment and tamoxifen therapy was terminated. Samples were obtained after 3, 24, 48, and 72 hours, as well as 1, 2, 3, 4, 5, and 6 weeks after the patients received their last doses.

Breast Cancer Task Force Criteria were used to judge response (Proposed Guidelines, Report from the Combination Chemotherapy Trials Working Group). To these criteria was added an improvement category, defined as < 50% but > 25% decrease in the sum of the products of the diameters of measured lesions.

## RESULTS

### Single Dose Kinetics

Figure 1 shows the tamoxifen pharmacokinetics in 17 patients after they received a single 10-mg/m² dose. The median time to achieve peak tamoxifen levels following a single dose was 6 hours; the median peak level following a single dose was 14 ng/ml. However, levels 3 hours after administration were not markedly different from those seen at 6 hours. Half-life could not be determined accurately from these data, but it was ≥ 6 hours. Additional tamoxifen and desmethyltamoxifen levels were measured (in two patients receiving 10 mg/m²; in two patients receiving 20 mg/m²; and in one patient receiving 40 mg/m²) at 3, 12, 24, and 48 hours after receipt of an initial single dose. Half-life appeared to be approximately 9 hours after peak levels were reached, but could not be assessed accurately (table 1).

### Tamoxifen Levels After Continuous Administration at 10 mg/m² Twice Daily

Twelve patients received tamoxifen at the 10-mg/m² twice daily dose level continuously to the long half-life, it was not surprising that, 4 weeks after continuous administration, tamoxifen blood levels were ten times greater than those seen following a single dose (fig. 2). Mean steady-state values were not obtained until the patient had received tamoxifen for 16 weeks; these were 260 ng/ml.

### Tamoxifen Blood Levels and Response

Figure 3 shows no difference in tamoxifen blood levels between responders and nonresponders at the 10-mg/m² twice daily level. In this group of patients, the median time to response was 6 weeks. The lowest tamoxifen level in a responder at this time was 70 ng/ml. The mean value in responders was 180 ng/ml. The lowest steady-state value in a responder was 150 ng/ml. After 4 or more weeks of continuous administration, there were no differences between 3- and 12-hour tamoxifen blood levels.

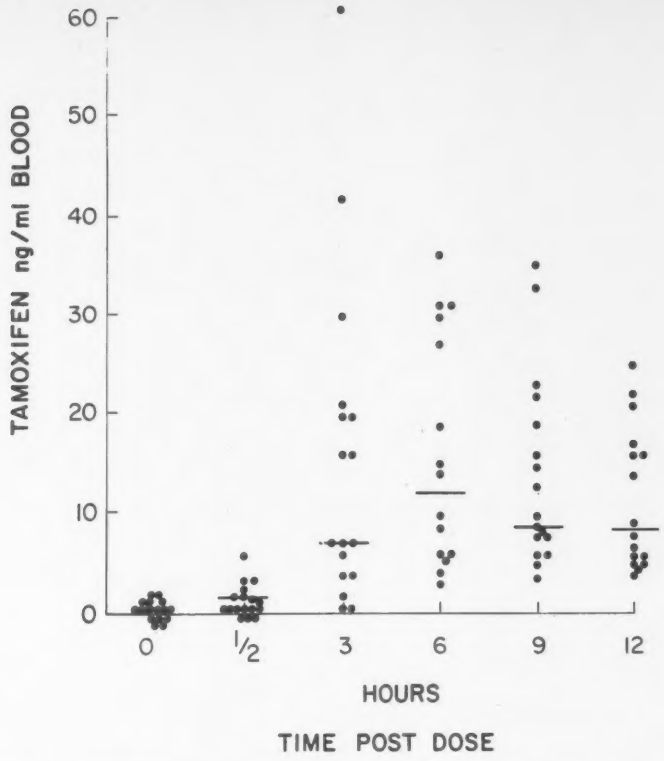


FIGURE 1.—Tamoxifen levels following an initial single dose of 10 mg/m² in 17 patients.

## Loading Dose Schedules

From the data obtained in the first 23 patients, it was clear that tamoxifen blood levels after a single dose were considerably lower than those obtained after continuous administration. It was unclear, however, how quickly levels compatible with response were achieved. Consequently, we began sampling at 3, 12, and 96 hours, and then weekly for the first 8 weeks of tamoxifen treatment. Mean values, so obtained in four patients treated at 10 mg/m², twice daily, are seen in figure 4. The previously observed mean value (180 ng/ml) for responders at 6–8 weeks was not obtained until 4–6 weeks of study, although the lowest value (70 ng/ml) previously observed in a responder was seen by Day 7 in a study in three of four patients.

In an effort to achieve blood levels known to be compatible with response in a shorter time, tamox-

TABLE 1.—Single dose values of tamoxifen and desmethyltamoxifen (ng/ml of whole blood)

| Time after administration | 10 mg/m² | | 20 mg/m² | | 40 mg/m² | |
|---|---|---|---|---|---|---|
| | Tamoxifen | Desmethyltamoxifen | Tamoxifen | Desmethyltamoxifen | Tamoxifen | Desmethyltamoxifen |
| 3 hours | 38 | 0 | 62 | 10 | 154 | 23 |
| 12 hours | 27 | 16 | 32 | 7 | 64 | 13 |
| 24 hours | 16 | 2 | 23 | 4 | 48 | 12 |
| 48 hours | 3 | 2 | 26 | 9 | — | — |

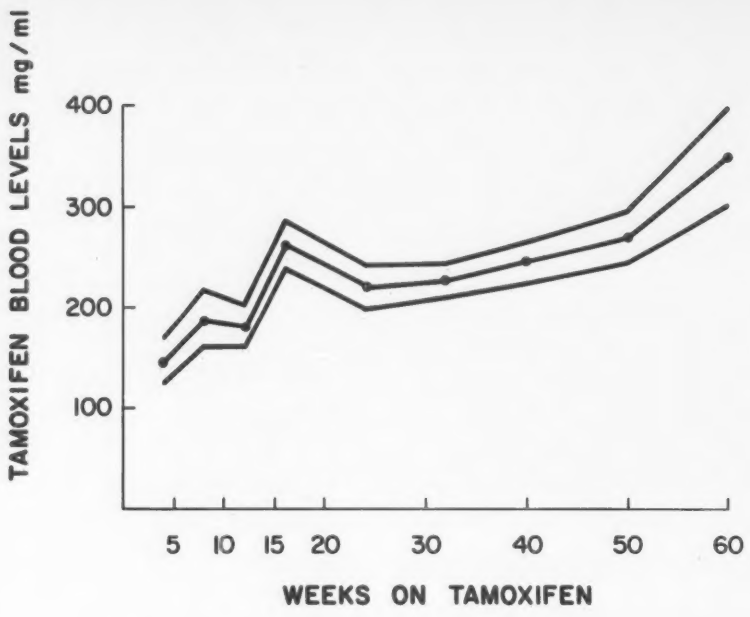


FIGURE 2.—Mean tamoxifen blood levels after a continuous dose of 10 mg/m², twice daily.

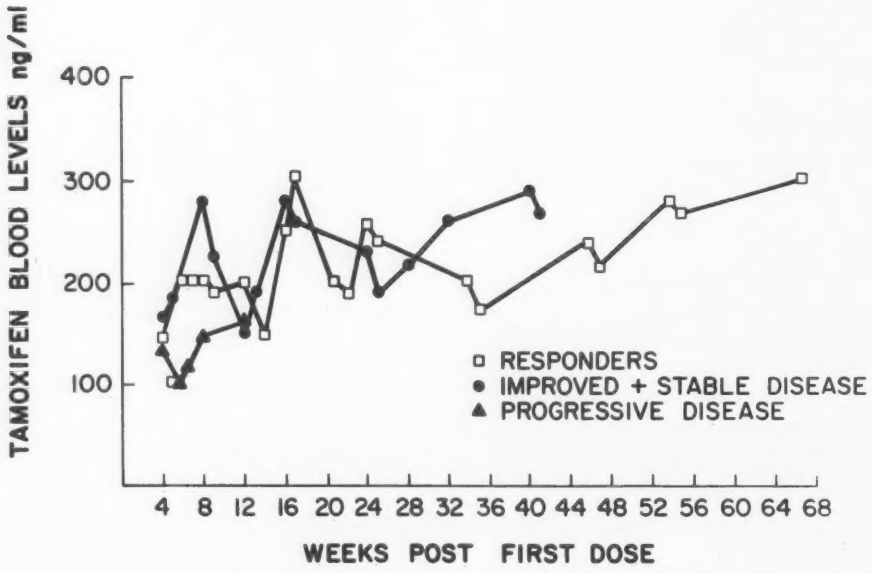


FIGURE 3.—Mean tamoxifen blood levels after continuous dose, by response category.

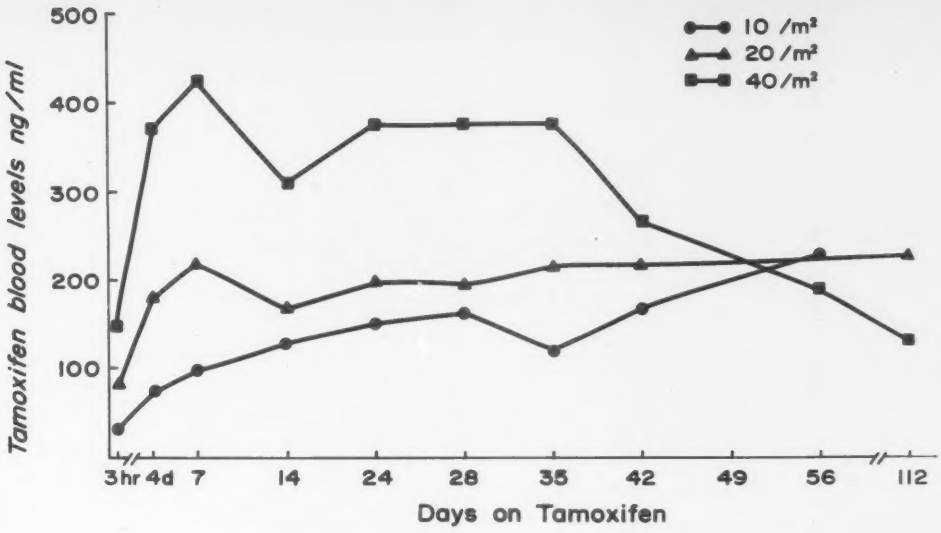


FIGURE 4.—Comparison of mean tamoxifen blood levels—10 mg/m² twice daily, and 20 and 40 mg/m² twice daily loading doses.

ifen was given as a loading dose for 1 week, as described earlier. After 7 days, the usual dosage of 20 mg/m² day was administered. Tamoxifen levels were measured for loading dose schedules in six patients receiving 20 mg/m² twice daily; in seven patients receiving 40 mg/m² twice daily; and in two patients receiving 80 mg/m² twice daily (fig 4). For the 40-mg/m² twice daily dose schedule, blood levels known to be associated with response (ie, ≥ 150 ng/ml) were present 3 hours after the first dose. The 80-mg/m² twice daily patients are not graphed. They achieved levels of 225 ± 10 ng/ml 3 hours after receiving the initial dose.

## Single Daily Dose Schedule

Because tamoxifen has such a long half-life and because no differences appeared between 3- and 12-hour values with a twice daily dose, patients were given 20 mg/m² of tamoxifen once daily. If adequate blood levels could be sustained when tamoxifen was given once daily, then patient compliance might theoretically be improved. Tamoxifen blood levels were measured in eight patients treated with 20 mg/m² as a single dose. Figure 5 compares 3- and 24-hour values to mean values obtained with the 10-mg/m² twice daily dose. There were differences in the 3- and 24-hour samples but, after 8 weeks of study, the 24-hour (nadir) values were within the therapeutic range.

## Comparison of Response and Toxicity by Dose Schedule

Of the 64 patients entered in the study, 57 are evaluable for response. Five are too early to evaluate, and two patients did not have measurable disease. Response rates by dose schedule are shown in table 2. Response rates on the standard 10-mg/m² twice daily dose schedule and the 20-mg/m² single daily dose schedule are similar. Only one response was seen in the 20 patients with the loading dose schedule, as opposed to in 13 of 37 patients receiving a total of 20 mg/m²/day. However, a lower percentage of the loading dose patients were ER+. In addition, the only evaluable site of disease in the majority of the patients receiving a loading dose was bone. Bone lesions are often difficult to evaluate, resulting in many patients being termed improved or as having stable disease. Importantly, the number of patients with progressive disease was similar: 15 of 37 of the patients without loading doses and 12 of 20 of the patients with loading doses. Toxicity comparison of the various dose schedules is shown in table 3. There did not appear to be an

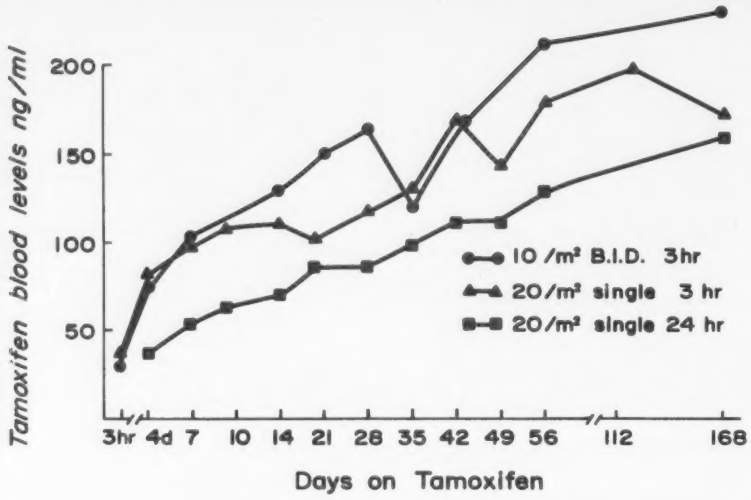


FIGURE 5.—Comparison of mean 3- and 24-hr tamoxifen blood levels in patients receiving a 20-mg/m² single daily dose.

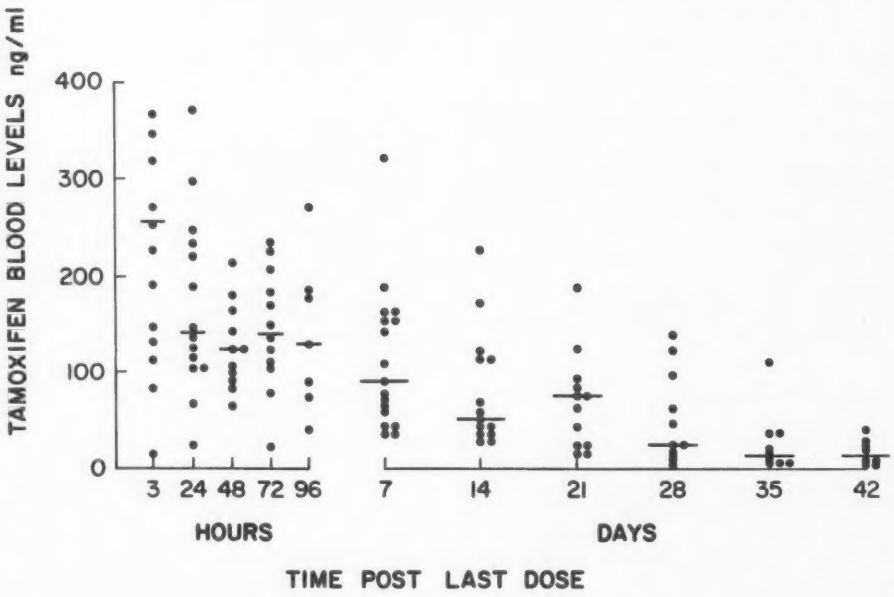


FIGURE 6.—Tamoxifen blood levels after discontinuation of continuous tamoxifen administration.

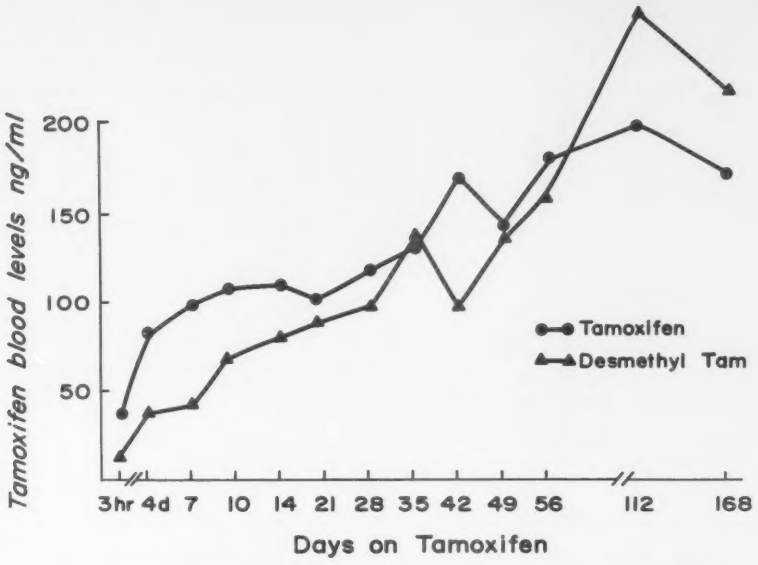


FIGURE 7.—Comparison of tamoxifen and desmethyltamoxifen mean blood levels in 8 patients treated with tamoxifen at 20 mg/m² (single daily dose).

appreciable difference between the loading and non-loading dose schedules.

### Tamoxifen Blood Levels Following Discontinuation of Drug After Continuous Dosing

Tamoxifen blood levels were drawn serially in 17 patients following discontinuation of tamoxifen therapy (fig 6). Median half-life was 7 days, with a wide range of 3–21 days. At 21 days, however, at least half of the patients had levels ≥ 70 ng/ml which, in at least one patient, was associated with a response. Tamoxifen was still detectable up to 6 weeks after discontinuation of the drug, although at a very low concentration.

### Metabolites

Two major metabolites of tamoxifen, desmethyltamoxifen and 4-hydroxytamoxifen (4-OH T) were also measured in some patients. Figure 7 shows desmethyltamoxifen levels, as compared with tamoxifen levels in eight patients treated with a 20-mg/m² single daily dose. Although mean desmethyl levels are initially approximately 50% of the tamoxifen levels, by 21 days levels are quite similar. Figure 8 shows desmethyl levels after discontinuation of continuous tamoxifen administration. The desmethyl metabolite followed a pattern of decay identical to that of the parent compound.

In one patient receiving 10 mg/m² twice daily, 4-OH T levels were measured from the inception of tamoxifen administation, along with tamoxifen and desmethyltamoxifen levels, for the first 7 weeks of dosing. Levels of 4-OH T were < 1 ng/ml the first week. Thereafter they increased slowly, but tamoxifen levels were always 15–25 times greater than 4-OH T levels. Steady-state levels of 4-OH T measured in three patients were approximately 20 ng/ml. This level is 15 times less than parent drug steady-state levels in all patients treated at 10 mg/m² twice daily.

## DISCUSSION

It is of interest that mean tamoxifen levels at the time responses were observed (6–8 weeks) were ten times higher (180 ng/ml) than peak levels after a single dose at the 10-mg/m² twice daily dose level (14 ng/ml). Using this dose schedule, the mean value for responders was not obtained until 4–6 weeks of study although the lowest value in a responder (70

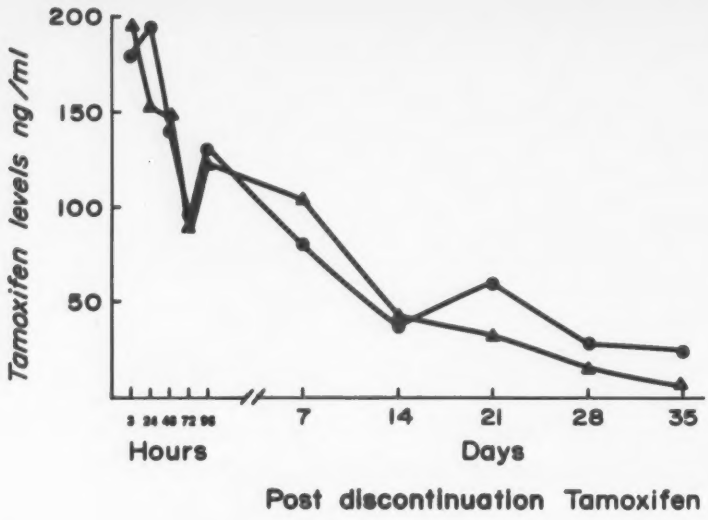


FIGURE 8.—Comparison of tamoxifen and desmethyltamoxifen mean blood levels after discontinuation of continuous administration of tamoxifen.

ng/ml) was observed in 75% of the patients by Day 7 of the study. Using a loading dose schedule of 40 mg/m² twice daily × 7 days, and then a 20-mg/m² single daily dose thereafter, levels of ≥ 150 ng/ml were obtained within 3 hours. However, in this small group of patients, there appeared to be no response advantage to the loading dose schedule. Determination of whether a loading dose schedule would result in a shorter time to response than a standard regimen of 10–20 mg twice daily would require a larger, randomized study.

It appears safe to give tamoxifen on a once daily schedule, particularly after the first 8 weeks, when trough (24-hour) values have risen into the known therapeutic range. The similar response rate of 42% in 12 patients receiving the single dose regimen of 20 mg/m², compared with 32% in 25 patients receiving the 10-mg/m² twice daily regimen, supports the empirical observations based on blood levels.

Jordan (5) has noted that uterine cytoplasmic estrogen receptors in rats remain low up to 72 hours after a single dose of tamoxifen. In our study, at least half of the patients had tamoxifen levels ≥ 70 ng/ml 3 weeks after administration, which, in our study, were associated with a response. Median levels at 28 days were 25 ng/ml, and at 6 weeks were 10 ng/ml. To avoid false-negative cytoplasmic ER assays, the assay should perhaps be delayed 4–6 weeks after discontinuation of the tamoxifen therapy.

TABLE 2.—Response to tamoxifen by dose schedule

| | No. of patients with— | | % of patients with— | | |
|---|---|---|---|---|---|
| Dose regimen | Evaluable response | ER+ | Response | Improvement and stable disease | Bone involvement |
| 10 mg/m² BID* | 25 | 11 | 32 | 24 | 44 |
| 20 mg/m² single | 12 | 6 | 42 | 25 | 42 |
| 20 mg/m² BID load | 7 | 5 | 14 | 43 | 86 |
| 30 mg/m² BID load | 4 | 1 | 0 | 25 | 100 |
| 40 mg/m² BID load | 7 | 1 | 0 | 43 | 86 |
| 80 mg/m² BID load | 2 | 0 | 0 | 0 | 50 |

*Twice daily.

TABLE 3.—Comparison of toxicity with standard vs loading dose

| Dose regimen | Total No. of patients | No. of patients with— Hot flash | Nausea | Headache | Flare | % of patients with toxicity |
|---|---|---|---|---|---|---|
| 10 mg/m² BID* | 27 | 4 | 2 | 1 | 1 | 30 |
| 20 mg/m² single | 13 | 4 | 1 | 1 | — | 46 |
| 20 mg/m² BID load | 7 | — | 2 | 2 | 3 | 57 |
| 30 mg/m² BID load | 3 | — | 1 | 1 | — | 33 |
| 40 mg/m² BID load | 7 | 2 | 1 | — | 1 | 29 |

*Twice daily.

More work with the major metabolites is indicated to determine if response patterns can be correlated with high or low levels of metabolite. Jordan et al (6) have found, in rat uteri, that 4-OH T has 12.5 times more antiestrogenic activity than does tamoxifen. Thus, this metabolite may have therapeutic importance, even though it is found in relatively low levels in patients receiving tamoxifen.

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# Tamoxifen: An Overview of Recent Studies in the Field of Oncology

**John S. Patterson* and Linda A. Battersby[1]**

## SUMMARY

**The safety and efficacy of tamoxifen, administered as a single agent in the palliative treatment of advanced breast cancer, are well-established. The role of tamoxifen as part of combined modality treatment still needs to be fully investigated, as does its usefulness as an adjuvant to mastectomy. The use of tamoxifen for tumors other than those in the breast is as yet not clearly defined. Results currently available in these areas are reviewed here.**

[Cancer Treat Rep 64:775–778, 1980]

Tamoxifen citrate (Nolvadex, property of Imperial Chemical Industries, Ltd) is now widely used as a palliative therapy for advanced breast cancer in women. The first clinical studies of this application were initiated in 1969 in Manchester, England. Since that time, the drug has become available throughout the world, and its use as a single agent in this disease area has been extensively reviewed (1–3). The overall objective response rate to therapy (> 50% tumor regression) is about 32%, and side effects are generally mild (1).

Current trials in advanced breast cancer are seeking to improve response rates to treatment and duration of response by combining tamoxifen with other agents, either hormonal or cytotoxic. The role of tamoxifen in treating early breast cancer, as an adjuvant to mastectomy, is also under evaluation.

Some workers are investigating the possibility of broadening the therapeutic profile of this antiestrogen. There is a rationale to support the use of tamoxifen in the treatment of other cancers. Estrogen-binding activity has been reported in many tumors, including those in the prostate (4), endometrium (5), hypernephroma (6), malignant melanoma (7), ovary (8), rat pancreas (9), and gastrointestinal tract (10). There is evidence that some of these tumors may be hormone-responsive (11,12), but it is by no means clear that estrogen-binding capacity is in any way related to the ability of these tumors to respond to endocrine maneuvers.

Finally, a number of tumors are known to produce ectopic hormones (13), which can lead to pharmacologic effects at certain target organs. The use of antiestrogens to counteract the end-organ effects of ectopic estrogen production has been investigated.

This report reviews the various areas of current clinical research with tamoxifen and summarizes the results available to date.

## COMBINATION TAMOXIFEN THERAPY FOR ADVANCED BREAST CANCER

### Endocrine Combinations

It is an attractive hypothesis that the combination of various different endocrine therapies may improve overall response rates, without greatly increasing the toxicity of therapy. However, unlike combination cytotoxic therapy, this goal has not easily been attained. Various investigators have shown no advantage to combining tamoxifen with a prolactin-lowering agent (14), a low-dose progestin (15), or diethylstilbestrol (DES) (16). Only Tormey et al (17) have suggested, in a preliminary analysis of a study of tamoxifen with or without fluoxymesterone, that combining the two agents improves the response rate from 28% in the tamoxifen-only group to 45% in the combination group ($P > 0.1$). This finding remains unconfirmed, since no other combination studies have been reported using androgen therapy.

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### Cytotoxic Combinations

It may be postulated that the combination of an antiestrogen with single or multiple cytotoxic chemotherapy could alter the response rate to cytotoxic chemotherapy alone in one of two ways. Either the response rate would improve as a result of the antiestrogen acting on the endocrine-sensitive, slow-growing cells, or the response rate could be adversely affected if the antiestrogen synchronizes cells in a rest phase, thus protecting them from the cytotoxic agents. Initial studies suggest that the former seems to be the case. Heuson (18) and Bosche et al (19) documented high response rates of combined chemotherapy and tamoxifen in uncontrolled studies. Mouridsen et al (20) have recently reported an ongoing randomized, prospective European Organization for Research on Treatment of Cancer study, in which a 47% response rate with CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) alone was augmented to 67% when tamoxifen, at a dose of 20 mg twice daily, was added to the CMF regimen in a second group of patients. This additive effect of tamoxifen and chemotherapy is confirmed in a second study (21) in which dibromodulcitol and doxorubicin (Adriamycin) were given with or without tamoxifen.

It is important to establish whether the improved response rate seen with combinations of antiestrogen and cytotoxic therapies offers any advantage to patients, many of whom would receive these agents (and many others) in a sequential fashion during the natural course of the disease. A very important study is in progress with the Swiss Cooperative Cancer Group (22). Postmenopausal women are randomly selected to receive either concomitant tamoxifen and combination cytotoxic chemotherapy with prednisolone, or tamoxifen alone, followed by the same chemotherapy regimen—either in progression or after 6 weeks if there is no evidence of response. The most recent analysis from this group shows that there is no difference in response rate or survival between the sequential- and concomitant-therapy groups.

Glick et al (23,24) have administered tamoxifen alone as initial therapy for advanced disease. The responding patients were then randomly selected to continue receiving tamoxifen alone or to receive concomitant (CMF) chemotherapy. As yet, no benefit has been demonstrated from the addition of CMF. There is no difference in response rate or duration, relapse rate, or patient survival between the two groups.

### Adjuvant Therapy

A large number of studies are in progress utilizing tamoxifen as a single agent or combined with cytotoxic chemotherapy given as an adjuvant to mastectomy. However, only two studies have reported any figures. In both, the results are very preliminary, and insufficient time has elapsed for survival data to be available.

One report (25) discussed by Palshof at the Second European Organization for Research on Treatment of Cancer Breast Cancer Working Conference in the spring of 1979 presented data from an endocrine study. The trial design has been presented previously (26). The study was initiated in 1975 and recruitment was terminated in March 1978. It has the advantage of being one of the longest running studies to have made regular estrogen-receptor measurements, but the disadvantage of difficulties in the pathologic staging, in that no axillary node sampling has been performed. The study suggests delayed recurrences in both pre- (21% tamoxifen vs 35% placebo) and post- (24% tamoxifen and 37% placebo) menopausal women regardless of estrogen-receptor status. DES was also found to be effective in the postmenopausal group, but this treatment was poorly tolerated.

Hubay et al (27) also reported data at the same meeting. All patients received CMF chemotherapy and one half were randomly assigned to receive additional tamoxifen for 12 months. A lower relapse rate for CMF and tamoxifen compared to that of CMF alone (10% vs 38.5% at 30 months, $P = 0.02$) was reported only in patients with positive estrogen receptors.

As with the Palshof study, data after only 30 months of study must be regarded as preliminary, although both of these trials must considerably encourage others involved in prospective adjuvant studies.

## OTHER ENDOCRINE-DEPENDENT TUMORS

### Prostate

Prostatic carcinoma has long been regarded as an endocrine-sensitive tumor. Good subjective responses have been documented with orchidectomy (11) and DES (28), although objective evidence of response, even in stage D disease, is not easily obtained. Since both prostatic carcinoma and adenomata are said to contain estrogen receptors (4,29), and to respond to pharmacologic estrogen therapy, a trial of anti-estrogen therapy was felt to be justified on the premise that since the situation is analogous to the situation in females with breast cancer.

Only two prospective studies have been published (30,31), both in abstract form. Both investigators saw objective regressions in patients with late disease. Two further anecdotal reports (32,33) add supportive evidence, although a larger phase II study in less heavily pretreated men is probably needed to justify a phase III study. However, a more detailed report by Glick et al appears elsewhere in this volume.

### Endometrium

Large amounts of estrogen and progesterone receptors have been described in this heavily steroid-dependent tissue. Although an endometrial tumor might be expected to be the most sensitive to an antiestrogen, there is a paucity of published data on the effects of tamoxifen. Bonte (34) reported one complete response (CR) and two partial responses (PR) in nine women who had received prior therapy with medroxyprogesterone acetate. The duration of response was short (2–4+ months). Swenerton et al (35) also saw four responders among seven patients; Broens, Mouridsen, and Soerensen[2] saw one CR, one PR, one disease stabilization (NC), and two progressive patients in a small study. The CRs and PRs were considerably longer (18 and 9 months, respectively) than those seen by Bonte (2–4+ months). The preliminary evidence cited here suggests that a prospective phase II study could now by justified in patients with stage IV endometrial carcinoma.

### Renal Cell Carcinoma

A number of investigators have reported the presence of specific estrogen binding in renal tumors (6,36). Giuliani et al (36) reported objective responses to tamoxifen in metastatic disease to bone in two of three cases. However, there are now three well-documented phase II studies of this tumor that are considerably less promising. Al-Sarraf (37) reporting a Southwest Oncology Group study of 49 patients, documented only two PRs and 14 NC patients. He concluded that further study of tamoxifen in renal cell carcinoma should be limited to combination with other therapies. Glick et al (31), with two NC patients and no responders in 12 cases, and Mulder and Alexieva-Figusch (38), with two disease stabilizations and no responders in 23 evaluable patients, both concluded that tamoxifen is ineffective as a therapy for metastatic renal cell carcinoma. It would be hard to justify further evaluation of this antiestrogen in this disease area.

[2]Personal communication.

### Other Tumors

MacBeth et al[3] have failed to demonstrate a response of colonic and gastric tumors to tamoxifen; however, they were also unable to demonstrate specific cytoplasmic estrogen-receptor protein in any of the gastrointestinal tumors they assayed. In addition, they failed to demonstrate estrogen receptors in malignant melanoma or to confirm anecdotal evidence of response to tamoxifen in six patients with the disease.

### Ectopic Hormone Production

Two papers (13,39) have described tamoxifen-induced regression of painful and painless gynecomastia associated with a gonadotropin-secreting oat cell carcinoma of the lung. One patient also had regression of associated hypertrophic pulmonary osteoarthropathy. Although these syndromes are not common, the reports suggest that tamoxifen deserves further assessments in painful gynecomastia associated with ectopic hormone-producing tumors.

## CONCLUSIONS

The nonsteroidal antiestrogen tamoxifen has been shown to be an effective antitumor agent. Its efficacy as a single agent in the treatment of advanced breast cancer has led to a number of studies aimed at assessing its place among other therapies for this disease. Its potential as an adjuvant therapy will become clearer as current studies are completed.

Further clinical trial work is indicated in prostatic carcinoma and adenocarcinoma of the endometrium, but tamoxifen seems to offer little against renal cell carcinoma. More data are required about the drug's efficacy in other tumor areas before firm conclusions can be drawn. It does not seem to block the effects of ectopic hormone production from some lung tumors and, thus, may play a part in palliating carcinoma of the lung.

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# Antiestrogen-Induced Remissions in Premenopausal Women With Stage IV Breast Cancer: Effects on Ovarian Function[1]

**Andrea Manni* and Olof H. Pearson[2]**

## SUMMARY

**Tamoxifen (T) was given in doses of 40–120 mg/day to 11 premenopausal women with stage IV breast cancer. Objective remission occurred in five, the disease did not progress in one, and five failed to respond. Duration of remission is 19+ months. Five patients underwent ovariectomy after receiving T: of two who responded to T and then relapsed, one responded to ovariectomy; three who failed to benefit from T also failed to improve after ovariectomy. The effect of T on the menstrual cycle ranged from no effect to complete cessation of menses, usually observed in patients who received larger doses. T induced a marked rise in serum estrone and estradiol, reaching levels up to 2500 pg/ml; serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels remained in the normal premenopausal range or were slightly elevated. In two patients in whom amenorrhea was induced with larger doses of T, both serum estrogen and gonadotropin levels were elevated. We conclude that T is effective in treating premenopausal patients with stage IV breast cancer. Because of the stimulating effect of T on ovarian function, escalated doses of T or castration plus T may be necessary in those patients who respond to T.**

[Cancer Treat Rep 64:779–785, 1980]

The effectiveness of antiestrogen therapy in postmenopausal women with metastatic breast cancer is now well-established (1). In our opinion, the antiestrogen T (Nolvadex) (T, the property of Imperial Chemical Industries, Ltd) is indeed the initial treatment of choice for hormone-dependent breast cancer in this group of patients (2). The role of antiestrogen therapy in premenopausal women, however, remains to be fully elucidated; in particular, its potential ability to replace surgical ovariectomy, which is now the standard treatment for hormone-dependent breast cancer in menstruating women. Our preliminary observations (2) and those of other investigators (3,4) have shown that remissions can be obtained with T in some premenopausal patients with metastatic breast carcinoma. We wish to report here our experience to date with the use of T in 11 premenopausal women with stage IV breast cancer, with particular emphasis on the effects this compound has on the pituitary-ovarian axis in these patients.

## MATERIALS AND METHODS

Eleven consecutive premenopausal patients with evaluable metastatic breast cancer were treated with T, initially with an oral dose of 20 mg every 12 hours. Because this dose was usually ineffective in suppressing the menstrual cycle, it was subsequently raised in some patients to a maximum of 60 mg orally every 12 hours. At the start of therapy, all women were menstruating regularly, with the exception of two who had undergone hysterectomies: premenopausal status of these patients was

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assessed by serum determination of estrogens and gonadotropins. The characteristics of the patients are summarized in table 1. Objective remission is defined as > 50% reduction of measurable lesions or recalcification of osteolytic lesions, with no progression elsewhere and no new lesions appearing for at least 3 months. No progression is defined as < 50% reduction or < 25% progression of measurable lesions, with no new lesions appearing for at least 6 months. Failure is defined at > 25% progression of measurable lesions, or the appearance of new lesions after an adequate trial (at least 6 weeks).

When patients relapsed or failed to respond to T, we performed (when possible) a surgical ovariectomy in an attempt to establish whether or not palliation (beyond that possible with antiestrogen therapy) could be obtained with castration. In some patients, T therapy was continued after ovariectomy. Serum gonadotropins were measured by radioimmunoassays (5,6). Radioimmunoassays of serum estrone and estradiol were performed initially by Diamond Shamrock Medical Science Laboratories, Columbus, OH, and later by Nichols Institute, San Pedro, CA. Estrogen receptors (ERs) in the tumor were measured by a dextran-coated charcoal technique (7).

## RESULTS

### Antitumor Effect of Tamoxifen in Premenopausal Patients

As can be seen in table 1, 6 of 11 patients obtained significant benefit, with an average duration in excess of 1½ years, from antiestrogen therapy. It should be pointed out that our patients had advanced disease, as indicated by the fact that all but one had either bone or visceral dominant site of metastasis. Three of five ER+ patients responded, whereas none of 2 ER– or mixed ER status did. (Patient 10 had ER– in the primary lesion and low ER+ in a metastatic lesion.) No significant side effects were encountered, except for occasional nausea or hot flushes, usually transient. Five patients underwent ovariectomies after receiving T: three who initially had not responded to antiestrogen treatment also did not respond to the surgery; of two who had responded to T and then relapsed, one obtained further remission with castration. This patient's (No. 1) pulmonary metastasis had completely disappeared while she remained on a T regimen that lasted 10 months. When relapse occurred, bilateral ovariectomy again induced a regression in the pulmonary metastasis, and the patient continues in remission more than 4 years after castration and T therapy.

### Effect of Tamoxifen on the Menstrual Cycle

Table 1 summarizes the effect of T on menses. It is apparent that complete suppression of ovarian function is not necessary to obtain an antitumor effect (patients 1–4, 6). In an attempt to establish whether larger doses of T would induce amenorrhea, we escalated the dosage in some patients. No increase in side effects was noted with the dosages used. In one patient (No. 4) raising the dosage of T to 80 mg induced cessation of menses; her menses were still present (although delayed and of shorter duration) during the initial 2 months of treatment when she was taking 40 mg/day (fig 1). In another patient (No. 6), the dosage of T had to be raised to 100 mg/day before amenorrhea could be induced. This condition, however, lasted only 5 months, followed by resumption of irregular menses, which prompted further escalation of the dosage to 120 mg/day. At this dosage, she continued to menstruate irregularly for 12 more months, after which she again became amenorrheic for the last 4 months of treatment (fig 2). One patient (No. 7) became amenorrheic on a dosage of 40 mg/day. However, it is difficult to evaluate the effect of T on the menstrual cycle in this woman because she was only treated for 2 months before she underwent an ovariectomy for progression of her disease. Escalating the dosage of T did not induce amenorrhea in three patients (Nos. 2, 3, 9). It should be noted, however, that in two patients (Nos. 2, 9), the duration of treatment was short due to progression of the disease; in a third (No. 3) the dosage was never raised above 50 mg/day. In one patient (No. 11), in whom cessation of menses occurred at 40 mg/day, the effect of T could not be evaluated because of simultaneous administration of cytotoxic chemotherapy.

### Effect of Tamoxifen on Serum Estrogens and Gonadotropins

Data on serum estrogens and gonadotropins were available in all patients except one (No. 8). Figures 1 and 2 show the serum level of FSH, LH, estrone, and estradiol in the two patients (Nos. 4, 6) who became amenorrheic from an escalation of the dosage of T. In both cases, there was an initial phase in which serum estrogens were markedly elevated and serum gonadotropins were in the "normal" premenopausal range, followed by a later phase in which serum FSH and LH rose into the postmenopausal range in the presence of persistently elevated estrogens.

TABLE 1.—Results of T therapy in 11 premenopausal patients with metastatic breast cancer

| Patient No. | Age | Disease-free interval (mos) | Site of metastasis | ER (fm/mg) | Maximum dose/day | Menses | Duration of treatment (mos)* | Response | | Response to ovariectomy |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Type | Duration (mos) | |
| 1 | 46 | 108 | Lung | — | 40 mg | Irregular | 53+ (10) | Remission | 10 | Remission† |
| 2 | 33 | 8 | Skin, bone | 47.0 | 60 mg | Regular | 9 (4) | Remission | 4 | Failure† |
| 3 | 53 | 0 | Breast, bone, peritoneum | 11.5 | 60 mg | Irregular | 15 | Remission | 15 | — |
| 4 | 51 | 54 | Pleura | — | 80 mg | Irregular → absent | 29+ | Remission | 29+ | — |
| 5 | 49 | 0 | Pleura | 43.0 | 40 mg | Hysterectomy | 22 | Remission | 22 | — |
| 6 | 45 | 26 | Bone | — | 120 mg | Irregular → absent | 40 | No change | 40 | — |
| 7 | 46 | 16 | Bone | 31.4 | 40 mg | Absent | 9 (2) | Failure | — | Failure† |
| 8 | 36 | 48 | Lymph nodes | 6.7‡ | 40 mg | Regular | 2 | Failure | — | Failure |
| 9 | 48 | 45 | Bone, pleura | Negative‡ | 80 mg | Regular | 5 (3) | Failure | — | Failure† |
| 10 | 35 | 0 | Bone | Negative/ 7.4‡ | 40 mg | Hysterectomy | 2 | Failure | — | — |
| 11 | 32 | 7 | Lung, bone | — | 40 mg | Absent | 2 | Failure | — | — |

*Nos. in parentheses = No. of mos T was given prior to ovariectomy.
†Continued to receive T after ovariectomy.
‡Done 1–3 weeks after stopping T.

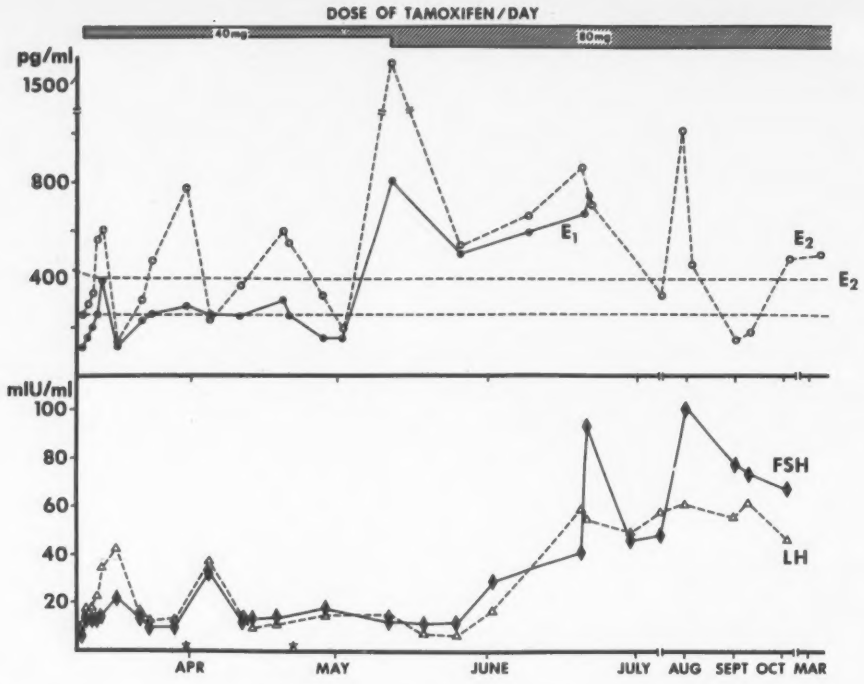


FIGURE 1.—Serum estrone ($E_1$) and estradiol ($E_2$) (upper panel) and FSH and LH (lower panel) in a premenopausal patient treated with escalating doses of T. Dotted lines indicate the upper limit of normal of $E_1$ and $E_2$ for midcycle peak. ★ menstrual period.

A marked rise in serum estrogens was observed in the majority of patients. Figure 3 (patient 7) shows that the rise in serum estradiol was very prompt after institution of T therapy. It can also be seen that, after ovariectomy and despite the continuation of T therapy, the expected fall in serum estrogens was observed. Figure 4 shows the rise in serum estradiol in another patient (No. 10) in the presence of fluctuating levels of serum gonadotropins, several of which are slightly above the normal premenopausal range. Serum estradiol level promptly fell after discontinuation of T therapy and institution of chemotherapy. Similar findings, already published (2), were present in patient 9. Patient 3 underwent a spontaneous menopause while receiving T, as shown by daily determinations of serum estrogens for 2 months. Her serum estrogens consistently rose into the postmenopausal range and serum gonadotropins also rose progressively. Sporadic determinations in patients 1, 2, and 5 also revealed elevated levels of serum estrone and estradiol, up to 500 pg/ml with fluctuating levels of serum gonadotropins between 10 and 30 mIU/ml. Patient 11 could not be evaluated because, as mentioned earlier, she received simultaneous chemotherapy. Frequent determinations of serum estrogens in this patient were very low, consistent with a castration effect from cytotoxic drugs.

## DISCUSSION

Our results, although limited to a small group of patients, indicate that antiestrogen therapy is effective in premenopausal women with metastatic breast cancer. T was able to induce remissions in a significant number of patients with advanced disease with an average duration indeed longer than that usually observed with ovariectomy. Also, the estrogen receptor status in the tumor seemed to be helpful in predicting response to antiestrogens, in line with its well-established predictive value of response to endocrine therapy in postmenopausal patients (8).

It became apparent to us early in the study that a T dosage of 40 mg/day, although able to induce tumor regression, was unable to completely suppress the menstrual cycle. This finding indicated that a complete antiestrogenic effect was not

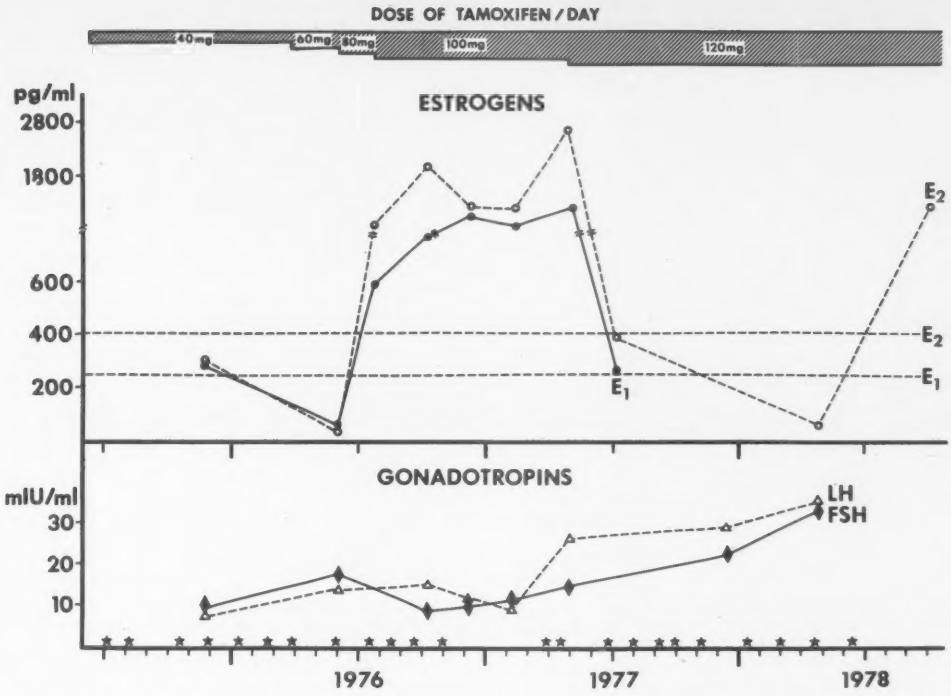


FIGURE 2.—Serum estrone ($E_1$) and estradiol ($E_2$) (upper panel) and FSH and LH (lower panel) in a premenopausal patient treated with escalating doses of T. Dotted lines indicate the upper limit of normal of $E_1$ and $E_2$ for midcycle peak. ★ menstrual period.

achieved, at least not at the level of the uterus. A possible explanation for this observation was the finding that T in premenopausal patients caused a marked rise in serum estrogens, which might in turn counteract (at least in part) the effect of T at the target tissue. Such stimulation of estrogen production by the ovaries in premenopausal patients is in contrast to the lack of effect of T on the ovaries in postmenopausal women—in whom we did not detect any change in serum estrone and estradiol during prolonged antiestrogen therapy (2). We believe that such stimulation is mediated through increased output of gonadotropins by the pituitary gland, which may sense a lack of estrogens in the presence of T. In two patients, we indeed demonstrated high levels of FSH and LH in the presence of elevated levels of serum estrogens. We believe that the "normal" or slightly elevated serum levels of gonadotropins observed in the other patients were indeed inappropriately elevated for the high circulating levels of estrogens. Of interest in this regard, is a recent report that studied the effect of continuous T administration on the pituitary-gonadal axis in men (9). The authors found a rise in serum gonadotropins, estrogens and testosterone—indicative of a stimulatory action of T on the pituitary-gonadal axis in men in agreement with our findings in women.

The increase in the serum level of estrogen is of concern because it is possible that it may at least in part counteract the action of T at the tumor level and, thus, decrease its antitumor activity. Thus, we escalated the dosage of T in some patients to determine whether more effective antiestrogen effects (that is, suppression of menstrual cycles) could be obtained with larger dosages. Although by doing this we induced amenorrhea in two patients, further experience is needed to determine whether larger doses of T can regularly suppress the menstrual cycle and completely counteract the increased estrogen secretion by the ovaries.

The response to ovariectomy after T therapy deserves some comment. Of special interest is patient 1, who had relapsed after T therapy and then ob-

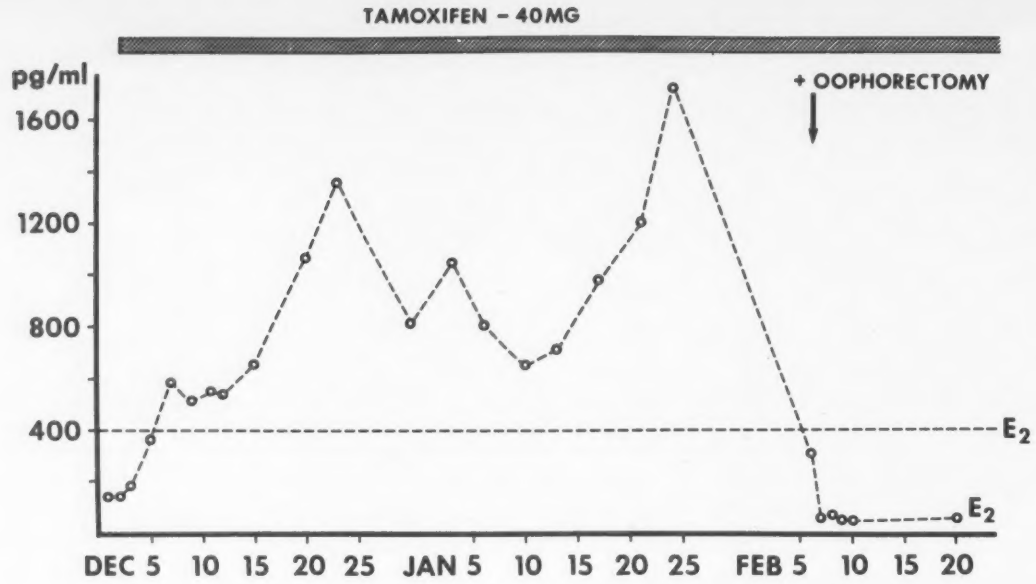


FIGURE 3.—Serum estrone ($E_1$) and estradiol ($E_2$) in a premenopausal patient treated with T. Dotted line indicates the upper limit of normal of $E_2$ for midcycle peak.

tained further remission after castration. These findings suggest that 40 mg of T daily in this patient were insufficient to block all estrogen action and that ovariectomy, by removing the major endogenous source of estrogens, permitted more effective antiestrogen action and tumor regression. Whether the same palliation would have been possible by simply raising the dose of T instead of by removing the ovaries remains, unfortunately, speculation. It is of interest that those premenopausal patients who failed to benefit from T therapy and subsequently underwent ovariectomies also failed to benefit from this ablative procedure. This finding is in agreement with the report by Pritchard et al (4), who also found that seven premenopausal patients who failed to respond to T therapy subsequently failed to respond to ovariectomies. If this finding is confirmed in a larger series of patients, then T might be used as the initial treatment of choice in premenopausal patients with hormone-dependent tumors. Those who failed to benefit would be treated with chemotherapy, without undergoing ovariectomies. Patients who improved with T could subsequently be treated by ovariectomies or radiation castration as a means of compensating for the increased estrogen secretion by the ovary induced by the antiestrogen.

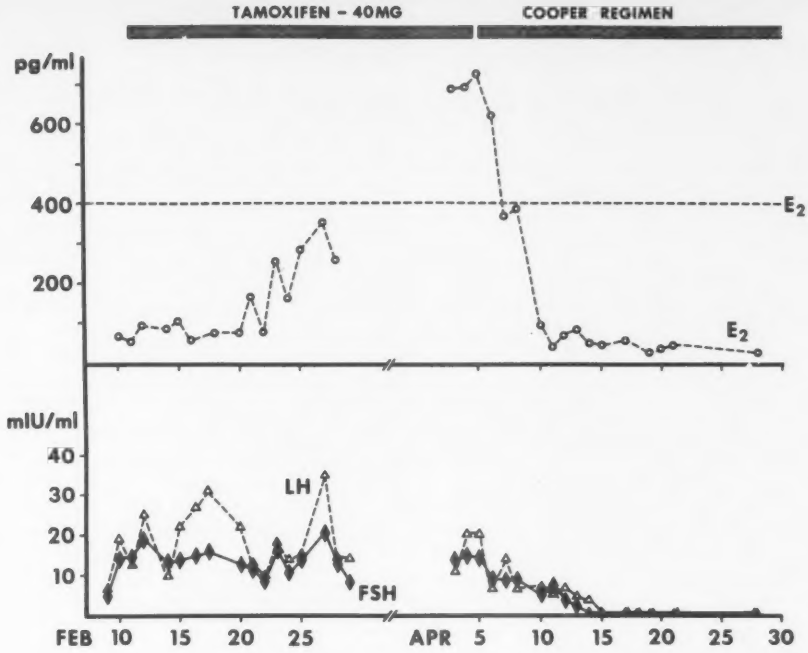


FIGURE 4.—Serum estradiol ($E_2$) (upper panel) and FSH and LH (lower panel) in a premenopausal patient treated with T. Dotted line indicates the upper limit of normal of $E_2$ for midcycle peak. On February 28, the patient was discharged from the hospital and readmitted on April 2.

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# Tamoxifen Therapy in Premenopausal Patients With Metastatic Breast Cancer[1]

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## SUMMARY

**Tamoxifen was evaluated in a phase II trial as initial hormonal therapy in premenopausal patients with metastatic breast cancer. The study design was such that responders remained on tamoxifen therapy; those who initially or subsequently progressed went on to ovarian ablation either by surgery or irradiation. Of 42 evaluable patients treated with tamoxifen, three had complete responses (CR), ten had partial responses (PR), and four remained stable (ST), giving total response rates of 32% (CR + PR) or 41% (CR + PR + ST). Among the 18 patients with positive estrogen (ER) or progesterone (PgR) receptors, there were eight responders, but only one responder (ST) in the nine patients with negative ER or PgR. Of the 25 patients who failed to respond to tamoxifen, 13 underwent ovarian ablation; all failed to respond. These 13 included four patients who were ER positive or equivocal and PgR positive or unknown. Nine tamoxifen responders (CR + PR + ST) have subsequently progressed; of these, eight have gone on to ovarian ablation. Six of these eight have responded (five PR and one ST) to ovarian ablation, and one has failed to respond. Thus, steroid receptors generally predicted a patient's response to tamoxifen therapy, but response to tamoxifen also strongly predicted a patient's subsequent response to ovarian ablation.**

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Classical teaching suggests a sequential approach in the management of patients with metastatic breast cancer. In premenopausal women who develop metastatic disease, the first form of hormonal treatment has usually been ovarian ablation either by surgical or radiotherapeutic means. Approxi-

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mately 30% of unselected patients respond to ovariectomy, and it is now recognized that, if estrogen receptor (ER) is present in the tumor, some 50%–70% of the patients will respond (1).

Tamoxifen has a structure very similar to that of diethylstilbestrol. It is a synthetic, nonsteroid compound with potent antiestrogenic activity in several mammalian species (2). Tamoxifen has been used mainly in the management of patients who are postmenopausal, and cumulative results from Britain and the US indicate an average objective response rate of 32% in unselected postmenopausal patients (3). In patients with positive ER, response rates are about 50% (3). Only a small number of patients with negative ER have responded to treatment with tamoxifen (3).

In spite of extensive testing in postmenopausal women, tamoxifen has received little attention in the treatment of premenopausal women with metastatic breast cancer. In theory, antiestrogens should have effects on breast tumors similar to those of ovariectomy. When this study was initiated in 1976, only 13 premenopausal women with metastatic breast cancer who had received tamoxifen were reported in the literature (4). Of these, five had had partial responses. There have been various reports, each describing a few additional premenopausal women treated with tamoxifen, since that time (5–10).[4]

This trial was designed, first, to assess the value of tamoxifen in treating premenopausal patients with metastatic breast cancer; second, to determine whether tamoxifen is equivalent to, or predictive of, response to ovariectomy; and third, to determine whether ER predicts response to tamoxifen in this group of patients (as it predicts response to other hormonal maneuvers).

## MATERIALS AND METHODS

### Patient Selection

Patients were categorized as premenopausal if their last menstrual period had occurred within 6 months of trial entry. Women with prior hysterectomies, but with one or both ovaries left intact, were included if they were < 50 years of age. Patients < 50 who had become amenorrheic while on chemotherapy were considered eligible if their menstrual periods had been regular until they began chemotherapy and if they had had no other hormonal manipulations. Thus, 49 patients with biopsy-proven breast cancer were entered in the study. All had metastatic disease, measurable either directly or radiographically. Patient age ranged from 28 to 52 years, with a median of 44. Individual patient characteristics are shown in table 1. No patient had received prior hormonal manipulation as systemic treatment for breast cancer, although five had been given brief courses of corticosteroids prior to trial entry: four (Nos. 8, 11, 15, 19) for brain metastases and one (No. 14) for radiation pneumonitis secondary to postoperative radiation therapy. Five patients (Nos. 8, 20, 34, 35, 39) had received prior chemotherapy, but in all cases it was discontinued at least 4 weeks prior to trial entry. Two patients (Nos. 34 and 39) had become amenorrheic while receiving chemotherapy. Twenty-two patients had received local postoperative radiotherapy following their primary surgery. Eleven patients had received radiotherapy for metastatic disease; four (Nos. 8, 11, 15, 19) to the whole head, five (Nos. 12, 29, 31, 35, 41) to bony secondaries, and two (Nos. 13 and 25) to nodal areas, either concurrent with tamoxifen therapy or at intervals of 1–6 months prior to trial entry. In all instances in which previous or concurrent radiotherapy was given, assessment of tamoxifen response was made on the basis of measurable tumor masses in areas other than those irradiated.

### Trial Design

The trial design was such that each patient would receive tamoxifen in an oral dose of 20 mg twice daily for a minimum of 4 weeks. Those patients who remained stable or responded were to continue tamoxifen therapy; those who failed to respond initially, or who responded and then had disease recurrence, were to discontinue tamoxifen and be treated with ovarian ablation either by surgery or irradiation. All patients who received at least 4 weeks of tamoxifen therapy were considered assessable.

### Dosage Schedule and Delivery

All but eight of the patients studied received the tamoxifen dosage outlined above. Patients 3 and 12 had their tamoxifen dosage escalated after 11 and 12 weeks respectively in unsuccessful attempts to induce response. Patients 35, 42, and 44 received doses of 10 mg two or three times daily for part of their treatment because of prescription error. Three patients had their dosage reduced to 10 mg twice daily because of side effects, discussed later.

Ovarian ablation by irradiation was accomplished in all cases by a megavoltage unit at a skin-to-source

[4]Band P. Personal communication.

distance of 80–120 cm, using 15 × 12 cm opposing fields centered 2 cm above the upper edge of the symphysis pubis and in the midline; 400 rads were given to the midplane (coronal) of the pelvis for 3 consecutive days, to a total dose of 1200 rads.

### Statistics

All $P$ values were calculated by the use of the Fisher's Exact Test.

### Case Evaluation and Followup

All patients were initially evaluated with a complete history and physical examination including a pelvic examination. Laboratory tests prior to tamoxifen therapy included hemoglobin, leukocyte, differential, and platelet counts; blood urea nitrogen (BUN), serum creatinine, serum alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), and serum calcium levels; chest roentgenogram; bone radionuclide scan; and, when indicated, liver and spleen radionuclide scans and bone roentgenograms. The diameters of measurable lesions were determined in two perpendicular planes. Followup included a complete physical examination with careful measurement of lesions every 4 weeks. Hemoglobin, leukocyte, differential and platelet counts and serum calcium levels were measured 1 week after tamoxifen therapy was started and at monthly intervals thereafter. Other tests were performed as indicated to monitor disease progression or regression.

### Steroid Receptor Assays

Estrogen (ER) and progesterone receptor (PgR) levels were measured in tumor tissue whenever possible. In patients who had not had these assays performed at the time of primary surgery, every attempt was made to obtain biopsy material when an assessable recurrence developed.

All receptor determinations (except those for patients 2, 21, and 43) were done in the laboratory of D. J. A. Sutherland, using the following method. Breast tumor tissue was pulverized at liquid nitrogen temperature; suspended at 0°C in TED buffer containing 10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiothreitol and 3 mM $NaN_3$; and sonicated for three 5-second pulses with a Polytron homogenizer at a setting of 6 (with 30-second intervals for cooling). The cytosol, free of supernatant lipid, was collected after ultracentrifugation at 100,000 $g$ for 60 minutes. The steroid receptor assays were performed in duplicate on freshly prepared cytosols and measured specifically displaceable binding of labeled steroid by a dextran-coated charcoal method. The labeled species were (2 nM) 17-β estradiol for ER and R 5020 for PgR. The nonlabeled competitors were U-11, 100 or 17-β estradiol for ER and R 5020 for PgR determinations. After incubation overnight at 0°C, free and loosely bound steroid was removed by incubation with dextran-coated charcoal (Norit A 10%, dextran T 70 0.05% in PBS, diluted 1:20 in TED buffer prior to use) for 15 minutes. After centrifugation to remove the dextran-coated charcoal, the difference between the counts bound without added unlabeled steroid (a) and counts bound with a 100-fold excess of nonlabeled competitor (b) was used to determine the amount of specific binding (a − b). This difference was expressed as femtomoles per milligram by normalization with the protein concentration in the individual cytosols. The percentage of total counts displaceable by the unlabeled steroid was also used to calculate a binding index (a − b)/a.

Because there is a linear relationship between the logarithm of the receptor concentration in femtomoles per milligram and the binding index with this method for both ER and PgR (fig 1), both measurements were used in determining whether a given cytosol contained significant levels of receptor. The arbitrary criteria used for patients in this study for receptor status were (*a*) receptor positive if the concentration of receptor was > 10 fmols/mg of cytosol protein and the binding index > 30; (*b*) receptor negative if receptor concentration was < 5 fmols/mg and/or the binding index < 20; (*c*) all other results were designated as equivocal.

The receptor assays for patients 2, 21, and 43 were carried out in the laboratory of B. G. Mobbs by incubation of replicate cytosol aliquots with a single saturating dose of tritiated steroid in the presence or absence of excess cold steroid to permit correction for low-affinity binding. Free and loosely bound steroid was removed with dextran-coated charcoal. High-affinity binding was expressed as femtomoles per milligram wet weight of tissue homogenized, and per milligram of DNA in the homogenate or pellet, except where the tumor specimen contained signficant numbers of nontumor cells. In these cases, the result was related to wet weight of tissue only. For ER, assay was carried out as described by Mobbs and Johnson (11). Briefly, the tumor cytosol was incubated with 1 nM $^3$H-estradiol (New England Nuclear, specific activity 85-105 curies/mmol) overnight at 4°C, in the presence or absence of 400 nM radioinert estradiol. Free and loosely bound hormone was removed by incubation with dextran-coated charcoal for 15 minutes at 0°C.

TABLE 1.—Patient characteristics and response to therapy

| Patient No. | Age | Race* | Disease-free interval (days) | Prior therapy† | Dominant disease site‡ | ER§ | PgR§ | Tamoxifen administration (days) | Response— Type¶ | Response— Time (days) | Response— Duration | Subsequent therapy** | Response— Type | Response— Duration (days) | Survival†† |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 38 | Cauc | 274 | S,R | Soft | − | ND | 70 | F | — | — | CMF | PR | 196 | 756+ |
| 2 | 39 | Cauc | 514 | S | Soft | + | ND | 118 | F | — | — | R A | F | — | 686 |
| 3 | 44 | Cauc | 5 | S | Visc | − | − | 132 | F | — | — | Ooph | F | — | 515+ |
| 4 | 38 | Cauc | 672 | S,R | Visc | − | − | 19 | F | — | — | CAMF | PR | 290 | 332 |
| 5 | 47 | Cauc | 435 | S,R | Soft | ND | ND | 392 | PR | 77 | 385 | R A, Pred | PR | 282+ | 746+ |
| 6 | 34 | Cauc | 1298 | S,R | Visc | ND | ND | 630+ | CR | 31 | 630+ | — | — | — | 630+ |
| 7 | 42 | Cauc | 1743 | S,R | Visc | ND | ND | 35 | F | — | — | Ooph | F | — | 269 |
| 8 | 39 | Cauc | 153 | S,C,D,R$^c$ | Soft | − | − | 7 | F | — | — | A | F | — | 57 |
| 9 | 33 | Cauc | 1378 | S,R | Visc | + | + | 705+ | PR | 29 | 705+ | — | — | — | 705+ |
| 10 | 45 | Cauc | 990 | S | Bone | ND | ND | 703+ | PR | 175 | 703+ | — | — | — | 703+ |
| 11 | 47 | Cauc | 103 | S,R,R$^c$,D | Visc | ND | ND | 217 | ST | — | 182 | R A | F | — | 546 |
| 12 | 28 | Cauc | 20 | S,R$^o$ | Soft | + | + | 94 | PR | 29 | 85 | Ooph | PR | 182 | 590+ |
| 13 | 46 | Cauc | 918 | S,R,R$^o$ | Soft | − | − | 28 | F | — | — | Ooph | F | — | 310 |
| 14 | 51 | Cauc | 422 | S,R,D | Visc | ND | ND | 43 | NA | — | — | Ooph | CR | 556+ | 599+ |
| 15 | 41 | Cauc | 2192 | S,D,R$^c$ | Visc | ND | ND | 30 | F | — | — | CMF | ST | 84 | 134 |
| 16 | 34 | Cauc | 28 | S | Visc | + | + | 270 | PR | 56 | 203 | Ooph, Pred | PR | 326+ | 599+ |
| 17 | 43 | Cauc | 1034 | S,R | Soft | ND | ND | 27 | F | — | — | Ooph | F | — | 232 |
| 18 | 48 | Cauc | 777 | S | Bone | ND | ND | 66 | NA | — | — | Cy, Pred | NA | — | 583+ |
| 19 | 39 | Cauc | 960 | S,R,D,R$^c$ | Visc | ND | ND | 45 | F | — | — | CMF | ST | 111 | 399 |
| 20 | 37 | Cauc | 540 | S,R,C | Visc | ND | ND | 28 | F | — | — | R A, FAC | F | — | 53 |
| 21 | 48 | Cauc | 420 | S | Bone | ± | ND | 554+ | ST | — | 554+ | — | — | — | 554+ |
| 22 | 48 | Cauc | 1822 | S,R | Visc | ND | ND | 267 | CR | 58 | 210 | R A, Pred | ST | 179 | — |
| 23 | 40 | Cauc | 1886 | S,R | Visc | ND | ND | 21 | NA | — | — | Not known | — | — | 552+ |
| 24 | 48 | Cauc | 0 | S | Bone | + | + | 310 | NA | — | — | Ooph | F | — | 477 |
| 25 | 43 | Cauc | 925 | S,R,R$^o$ | Soft | + | + | 91 | F | — | — | R A, Pred | F | — | 536+ |
| 26 | 40 | Cauc | 469 | S | Visc | ND | ND | 34 | F | — | — | CF | F | — | 67 |
| 27 | 29 | Cauc | 177 | S | Visc | ± | + | 77 | F | — | — | Ooph | F | — | 113 |
| 28 | 51 | Cauc | 2678 | S,R | Visc | ND | ND | 462+ | PR | 57 | 462+ | — | — | — | 462 |
| 29 | 34 | Cauc | 16 | S,R$^o$ | Bone | ND | ND | 9 | F | — | — | Ooph | F | — | 330 |
| 30 | 39 | E Ind | 0 | S | Visc | − | + | 43 | F | — | — | MDP | F | — | 82+ |
| 31 | 46 | Cauc | 2389 | S,R$^o$ | Visc | ND | ND | 177 | PR | 56 | 168 | R A, CMF | PR | 308+ | 489+ |
| 32 | 46 | Cauc | 704 | S | Soft | − | + | 28 | ST | — | 28 | Ooph | PR | 354 | 392+ |
| 33 | 50 | Cauc | NA | S,R | None | ND | ND | 14 | NA | — | — | R A | NA | — | 406+ |
| 34 | 46 | Cauc | 895 | S,R,C | Bone | ND | ND | 168 | F | — | — | Ooph | F | — | 392+ |
| 35 | 52 | Cauc | 1306 | S,R,R$^o$ | Visc | ND | ND | 144 | F | — | — | Ooph | F | — | 396+ |
| 36 | 39 | Cauc | 202 | S,C | Soft | − | ND | 18 | NA | — | — | Ooph, A V, surg | NA | — | 238+ |
| 37 | 46 | Cauc | 267 | S | Soft | ND | ND | 35 | F | — | — | Ooph | F | — | 268 |
| 38 | 44 | Negro | 654 | S | Soft | ± | + | 204 | PR | 28 | 204 | R A | PR | 174+ | 379+ |
| 39 | 44 | Cauc | 994 | S,C | Visc | − | + | 14 | F | — | — | Died | — | — | 20 |
| 40 | 41 | Cauc | 452 | S,R | Visc | ND | ND | 287+ | CR | 28 | 287+ | — | — | — | 259+ |
| 41 | 50 | Cauc | 0 | S,R$^o$ | Bone | + | + | 259+ | F | — | — | — | — | — | 259+ |
| 42 | 46 | Cauc | 756 | S,R | None | ND | ND | 259+ | NA | — | — | — | — | — | 259+ |
| 43 | 44 | Negro | 1541 | S | Bone | + | ND | 329 | PR | 182 | 323 | Ooph | TE | — | 330+ |
| 44 | 48 | Cauc | 1359 | S | Soft | + | + | 289+ | PR | 93 | 289+ | — | — | — | 289+ |
| 45 | 49 | Cauc | 182 | S,R | Visc | + | + | 17 | F | — | — | Died | — | — | 17 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 46 | 45 | Orien | 1435 | S | Bone | ND | ND | 140 | F | — | — | CMF | ST | 63+ | 203+ |
| 47 | 33 | Cauc | 686 | S | Soft | ND | ND | 61 | F | — | — | CMF | TE | — | 184+ |
| 48 | 39 | Orien | 318 | S,R | Visc | + | + | 28 | F | — | — | RA | F | — | 154+ |
| 49 | 46 | Cauc | 139 | S | Bone | + | + | 213+ | ST | — | 213+ | — | — | — | 213+ |

*Cauc = Caucasian; E Ind = East Indian; Orien = Oriental.

†S = surgery; R = local postoperative radiotherapy; C = chemotherapy; D = dexamethasone or other corticosteroids; $R^c$ = cranial radiotherapy; $R^o$ = radiation to other sites of metastatic disease.

‡Soft = soft tissue; visc = visceral.

§− = negative; + = positive; ND = not done; ± = equivocal.

¶F = failure; NA = not assessable; ST = stable.

**CMF = cyclophosphamide, methotrexate, and 5-FU; RA = radiation ablation of ovaries; Ooph = surgical oophorectomy; CAMF = cyclophosphamide, adriamycin, methotrexate, and 5-FU; Pred = prednisone; A = adriamycin; Cy = cyclophosphamide; FAC = 5-FU, adriamycin, and cyclophosphamide; CF = cyclophosphamide and 5-FU; MDP = medroxyprogesterone; AV = adriamycin and vincristine; TE = too early to be assessed.

††Survival is calculated from date tamoxifen was started.

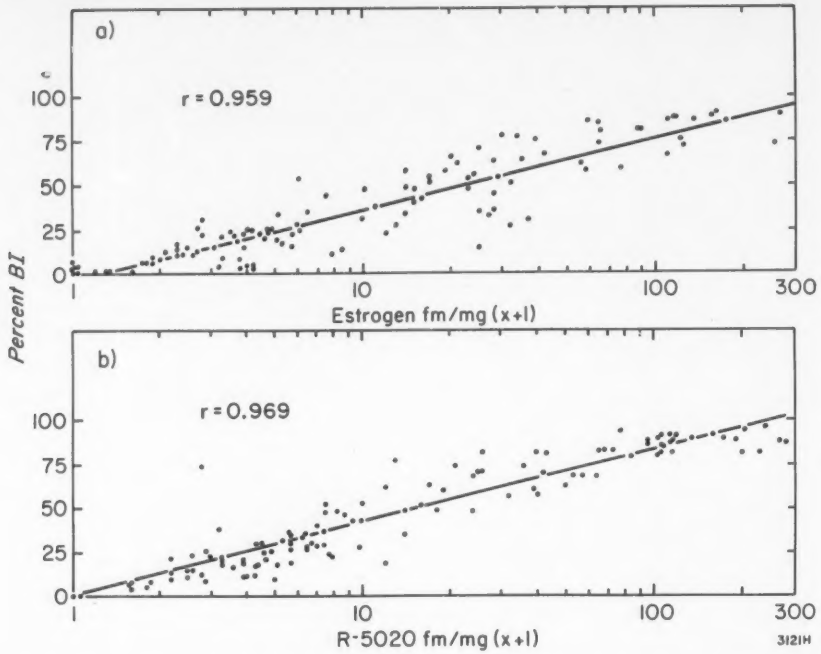


FIGURE 1.—% binding index (BI) as a function of receptor concentration expressed in femtomoles per milligram of cytosol protein for 17-β estradiol, (a), and for R 5020, (b). The r values and lines indicate the correlation coefficient and least squares fit of the BI and the log transform of the receptor concentrations.

The "cutoff" points used to distinguish receptor-negative from receptor-positive tumors were as follows: ER negative, tumors with < 0.27 fmols/mg and < 120 fmols/mg DNA; ER positive > 0.37 fmols/mg and > 180 fmols/mg DNA. Tumors with receptor values between these cutoff points, or classified as receptor-positive by one parameter (eg, wet weight), but receptor-negative by the other (eg, DNA content), were classified as borderline, (±). The ER cutoff points have been established by the relationship between clinical response and ER values in 17 evaluable pre- and perimenopausal patients treated by a variety of hormonal manipulations.

## Assessment of Tumor Response

Objective tumor responses were judged in a manner similar to that established by the Breast Cancer Task Force (12), as follows.

*Complete Response (CR):* Complete disappearance of all clinically detectable malignant disease for at least 4 weeks. Patients with bony metastases should have normalization of all bone roentgenograms.

*Partial Response (PR):* ≥ 50% decrease in tumor size for at least 4 weeks, without increase in size of any area of known malignant disease or appearance of new areas of malignant disease. A partial response of bony metastases occurs if there is a partial decrease in size of lesions, blastic transformation of lytic lesions, or decreased density of blastic lesions, lasting for at least 4 weeks.

*No Change (ST):* < 50% decrease or < 25% increase in tumor size or no significant change in evaluable disease for at least 4 weeks (≥ 8 weeks for bony metastases).

*Progression:* ≥ 25% increase in size of measured lesions present at the start of therapy, or a definite increase in size of evaluable lesions present at the start of therapy, or appearance of new metastatic lesions known not to be present at the start of therapy.

Bone scans were used to document the presence or absence of tumors, but not to evaluate disease response.

TABLE 2.—Response to tamoxifen vs hormone receptor status

| Response | ER+/PgR+ | ER+/ PgRND* | EREq†/PgR+ | EREq/PgRND | ER−/PgR+ | ER−/PgRND | ER−/PgR− | ND/ND |
|---|---|---|---|---|---|---|---|---|
| CR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| PR | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 4 |
| ST | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| F‡ | 4 | 1 | 1 | 0 | 2 | 1 | 4 | 12 |
| NA‡ | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 5 |
| Totals | 10 | 2 | 2 | 1 | 3 | 2 | 4 | 25 |

*ND = not done.
†Eq = borderline.
‡F = failure and NA = not assessable.

## RESULTS

### Antitumor Effect

Of 49 patients registered in the study, seven were inevaluable. Of these, two (Nos. 18 and 24) had bone scans as their only evidence of metastatic disease, and two (Nos. 33 and 42) had no residual metastatic disease whatsoever. Patients 23 and 36 were withdrawn from tamoxifen therapy after 19 and 21 days respectively, not because of disease progression or side effects, but to pursue a form of therapy they or their referring doctors preferred (meditation in one case and surgical ovariectomy in the other). Both of these patients had been stable with tamoxifen until their withdrawal, but were excluded from evaluation because of their short treatment period and elective withdrawal from therapy. A seventh patient (No. 14) could not be evaluated because the lung lesions seemed to be a mixture of malignant and inflammatory disease. Chest roentgenogram showed fluctuation in size and position in an inexplicable fashion. Five other patients (Nos. 4, 8, 29, 39, and 45) who received tamoxifen for < 4 weeks are included with the assessable patients as failures. They were withdrawn from tamoxifen therapy because of rapidly progressive disease.

Of the 42 evaluable patients, three had CR, ten had PR, and four had ST, giving total response rates of 32% (CR + PR) or 41% (CR + PR + ST). Response rates in relation to hormone receptor status are shown in table 2. The response rate for ER+ patients is 5/11 (CR + PR) or 6/11 (CR + PR + ST); the response rate for ER− patients is 0/8 (CR + PR) or 1/8 (CR + PR + ST). The response rate for PgR+ patients is 5/14 (CR + PR) or 7/14 (CR + PR + ST); the response rate for PgR− patients is 0/4.

CR occurred only in patients with ascites (No. 40) and pleural effusions (Nos. 6 and 22). PR occurred in patients with major sites of metastatic disease in soft tissue or nodes (Nos. 5, 12, 38, and 44), lung (Nos. 9 and 31), liver (No. 16), bone (Nos. 10 and 43), and pleural effusion and nodes (No. 28). Patients remaining stable had metastatic disease in bone (Nos. 21 and 50), liver and gastrointestinal tract (No. 11), and soft tissue (No. 32).

The amount of time for a definite response to be noted ranged from 4 weeks in patients with visceral or soft tissue disease to 26 weeks in one patient with bony secondaries. Ten patients with progressive disease discontinued tamoxifen therapy after ≤ 4 weeks of treatment; there is no way of knowing if any of these patients would have eventually responded to continued tamoxifen therapy.

Response duration for patients with CR and PR ranged from 85 to 705+ days; the median response duration will be at least 289 days, but the final median has not yet been ascertained. The response duration for the four patients with ST was 28–544+ days, with a median response duration of at least 193 days.

There was no significant correlation between age (≥ 45 vs < 45) and response rate ($P > 0.1$), between disease-free interval and response rate ($P > 0.1$) or between age (≥ 45 vs < 45) and the development of amenorrhea ($P > 0.2$). However, it seemed that patients who developed any sign or symptom of "estrogen deprivation" (hot flushes, altered menstruation, or amenorrhea) while receiving tamoxifen were more likely to respond to it ($P < 0.01$).

### Side Effects

Side effects, which were generally mild or minor, are shown in table 3. Nineteen of the 49 patients treated underwent menstrual cycle changes. Seven had minor alterations such as changes in frequency or amount of bleeding or single missed periods. Three had intermittent amenorrhea; nine underwent complete cessation of menstruation after intervals of 1–9 months. Seventeen patients complained of hot flushes or sweating (sometimes

TABLE 3.—Side effects in 49 premenopausal patients treated with tamoxifen

| Symptom | No. of patients |
|---|---|
| Any menstrual alteration | 19 |
| Total amenorrhea | 9 |
| Intermittent amenorrhea | 3 |
| Minor menstrual alterations | 7 |
| Hot flushes | 17 |
| Gastrointestinal side effects | 12 |
| Mental or CNS effects | 9 |
| Ovarian cysts | 5 |
| Hypercalcemia/possible disease flare | 4 |
| Rash | 4 |
| Generalized pruritus | 2 |
| Mild arthralgia/arthritis | 2 |
| Mild hirsutism | 2 |
| Transient constipation | 2 |
| Mild hair loss | 1 |
| Vaginal pruritus | 1 |
| Transient mild leukopenia | 1 |
| Transient mild thrombocytopenia | 1 |
| Serum sickness-like syndrome (patient receiving numerous other drugs) | 1 |

severe and frequent). Twelve patients had gastrointestinal side effects: anorexia (two); mild nausea (five); and nausea and vomiting (five). In three of the patients with nausea and vomiting, hypercalcemia developed concurrently and may have been responsible for the nausea. Nine patients complained of mental or central nervous system effects: mild depression or tearfulness (two); irritability (one); nervousness and tremor (two); and tiredness, light-headedness, and decrease in energy and concentration (one each).

In four cases, the possibility of hypercalcemia or disease "flare" due to tamoxifen was raised. These problems may have simply represented the natural progression of the uncontrolled metastatic breast cancer. In two of the three cases in which hypercalcemia developed, widespread bone metastases were already present at the time of study entry. In the third case, bone scan findings had been negative shortly before tamoxifen was given. Transient mild leukopenia occurred in one patient, and temporary mild thrombocytopenia in another. In both cases, tamoxifen was continued and the hematologic picture normalized within a few months.

Other side effects included the development of ovarian cysts in five patients; a mild transient acniform or maculopapular rash in four patients; and mild pruritus, slight increase in facial hair, and transient constipation in two patients each. Mild hair loss and vaginal pruritus each occurred in one patient. Two patients developed mild, otherwise unexplained arthralgia and/or arthritis during tamoxifen, and one patient underwent a severe serum sickness-like reaction while receiving tamoxifen and a number of other drugs.

Five patients had tamoxifen therapy discontinued because of possible side effects; four because of rapid disease progression (felt possibly to represent a disease "flare") and one because of nervousness and irritability after 4 weeks of tamoxifen. Three patients had their tamoxifen dosage reduced because of minor side effects (rash, nausea, tremor). All three patients were able to continue to receive 10 mg twice daily, without additional complications.

## Role of Tamoxifen in Predicting Response to Ovarian Ablation

Of the 25 patients who failed to respond to tamoxifen, 13 underwent ovarian ablation; all failed to respond. These 13 included two who were ER– and PgR– (Nos. 3 and 13); seven who were ER and PgR unknown (Nos. 7, 17, 20, 29, 34, 35, and 37); two who were ER+ and PgR+ (Nos. 25 and 48); one who was ER equivocal, PgR+ (No. 27); and one who was ER+, PgR unknown (No. 2). Eight of the 25 patients who failed to respond to tamoxifen were immediately treated with combination chemotherapy; two had PR (Nos. 1 and 4); three had ST (Nos. 5, 19, and 46); two failed to respond to chemotherapy (Nos. 8 and 26); and one has been treated too recently to be assessed (No. 47).

Nine tamoxifen responders (CR + PR + ST) have subsequently progressed and, of these, eight have gone on to ovarian ablation, five without any concurrent treatment (patients 11, 12, 32, 38, and 43) and three with the addition of prednisone (patients 5, 16, and 22). Six (five PR and one ST) of these eight patients have responded to ovarian ablation; one (No. 11) has failed to respond, and one (No. 43) is still too recently treated to be evaluated. The ninth patient (No. 31) was treated concurrently with ovarian ablation and combination chemotherapy and had a PR. Of the six responders to ovarian ablation, two (Nos. 12 and 16) were ER+ and PgR+; one (No. 38) was ER equivocal and PgR+; one (No. 32) was ER– and PgR+; and two (Nos. 5 and 22) were ER and PgR unknown. Patient 11, who responded to tamoxifen (ST) but failed to respond to ovariectomy, was ER and PgR unknown.

Thus, response to tamoxifen was highly predic-

tive of subsequent response to ovariectomy ($P < 0.002$). Indeed, in this group of patients, it seemed more strongly predictive than ER status.

## DISCUSSION

This study shows a response rate to tamoxifen of 32% (41% if patients with ST are included) in a group of premenopausal women with breast cancer. The group studied does not contain consecutive patients and has undoubtedly been "selected" in various ways. Within these limitations, however, the response rate to tamoxifen in this group of premenopausal patients seems comparable to that obtained with ovarian ablation in unselected premenopausal patients (1). The median response duration (which will be at least 289+ days) is also similar to that obtained with ovarian ablation (13,14). Furthermore, the response rate of these premenopausal women is similar to that of postmenopausal women treated with tamoxifen (3).

The mechanism of action of tamoxifen in controlling breast cancer has been somewhat controversial. Some researchers (15) have felt that tamoxifen acts by competing with estradiol for cytoplasmic receptor sites, thus blocking estrogen effect on the tumor cell. There is evidence, however, that tamoxifen may act by combining with receptors and forming tamoxifen-receptor complexes that are translocated into the cell nucleus and, in some way, cause reduced replenishment of cytoplasmic estrogen receptors (16).

In this study, it was noted that many patients who had tumor shrinkage while taking tamoxifen continued to have regular menstrual periods and showed no signs of estrogen deprivation. Further analysis of the data, however, suggests that patients who developed some sign or symptom of estrogen deprivation (hot flushes, menstrual alterations, amenorrhea) had a higher rate of response to tamoxifen ($P < 0.01$). In assessing this correlation, it is important to remember that, in patients who failed to respond to tamoxifen, therapy was discontinued after $\leq 8$ weeks, while those who responded to tamoxifen continued treatment for a longer time. Amenorrhea did not develop in most patients until after 3–9 months of tamoxifen therapy, and it is likely that this antiestrogenic effect may simply be related to treatment duration. In addition, it seems unlikely that, if tamoxifen were completely blocking estrogen activity, patients who relapsed while still receiving tamoxifen would then respond to ovarian ablation.

Our data suggest that patients who fail to respond to tamoxifen are unlikely to respond to ovarian ablation. It also seems that patients who respond well to tamoxifen have a very good chance of response to subsequent ovarian ablation. In fact, response to tamoxifen predicts response to subsequent ovariectomy more accurately than does estrogen receptor status. This finding is exemplified by patients 2, 25, 27, and 48. These patients had positive or unknown PgR and high or borderline ER, and would therefore have been considered likely to respond to ovarian ablation. Following their failure to respond to tamoxifen, however, these patients subsequently failed to respond to ovariectomy. If this pattern is consistent in a larger number of patients, those who fail to respond to tamoxifen might be spared ovarian ablations, regardless of their receptor status.

## CONCLUSIONS

This study shows that the response rate of premenopausal patients with metastatic breast cancer to tamoxifen is comparable to that of postmenopausal patients, as well as to the response rate of premenopausal patients to ovariectomy. ER and PgR predict response to tamoxifen, in that patients with low receptor levels are very unlikely to respond, whereas patients with high ER and/or PgR levels have an appreciable chance of response. Response to tamoxifen seems highly predictive of response to ovariectomy, both in a negative and a positive way.

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# Estrogen Receptors and Antiestrogen Therapy in Selected Human Solid Tumors

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## SUMMARY

**Tumor biopsies from a range of human cancers not normally thought to be hormone-dependent were assayed for the presence of both cytoplasmic and nuclear estrogen receptors, using strict criteria of specificity and affinity. Of 72 tumor samples assayed, only one had both cytoplasmic and nuclear receptors. However, several others showed the presence of estrogen-binding components with characteristics different from those of the normal human estrogen receptor. Thirty patients with advanced, measurable disease were treated with the antiestrogen tamoxifen. In only 25 of these was the response evaluable, and in each case, the disease progressed. This study suggests that the occurrence of the classic estrogen receptor in human cancers, other than breast and uterine, is rare; this finding is supported by the absence of response of advanced disease to antiestrogen treatment.**

[Cancer Treat Rep 64:797–799, 1980]

Additive endocrine therapy for cancer treatment has the two advantages of ease of administration and lack of complicating side effects (1). However, identification of those patients who will respond to endocrine therapy has proved difficult (2). Even the best of recent results suggest that only 75% of those patients predicted to respond to endocrine therapy will actually do so (3). The most successful index of potential response to hormone therapy for patients with breast cancer has been the identification of functional steroid receptors in a biopsy specimen (4,5). Given the poor results achieved with current chemotherapeutic regimens in treating some of the solid tumors and the recent suggestions that colon (6) and renal (7) cancers and malignant melanoma (8) might, in fact, contain an estrogen-binding component, the following study was undertaken to determine whether functional estrogen receptor could be identified in biopsies of selected cancers, not normally considered responsive to hormone therapy. The study was aimed specifically at determining the presence of functional estrogen receptor with the same characteristics of specificity and affinity as that found in normal endocrine tissue and in breast cancer. However, the possible existence of other, lower affinity estrogen-binding components was also considered. A group of patients with advanced, measurable cancer were also followed during treatment with the antiestrogen tamoxifen.

## PATIENTS

All patients, with the exception of those with malignant melanoma, were biopsied during surgery for primary disease and parallel specimens were taken for pathologic examination and for steroid receptor concentration measurement. Patients with malignant melanoma had biopsies of secondary disease. Only one of these patients had received prior chemotherapy, and he had been given a pulse of treatment 3 days before biopsy. There had, however, been obvious progression of disease during the 2 weeks after the preceding pulse, and the tu-

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mor was obviously insensitive to that particular regimen. Patients have not been differentiated by sex, but the study contains approximately equal numbers of males and females.

For the study of response to tamoxifen therapy, all patients were attending the Department of Clinical Oncology Clinic and all had advanced, measurable disease at the time of entry into the study.

## RECEPTOR STATUS

No attempt is made in this paper to assess patients by quantitative values of receptor concentration. Patients are reported simply as having measurable receptor in a particular fraction (+), using the criteria given below. Patients with no measurable receptor are reported as 0.

Steroid receptor content was determined for 72 biopsies. Each biopsy was fractionated into soluble and pellet fractions, and estrogen receptor concentration determined as reported previously (9). The following criteria were used to determine if an assay was positive for estrogen receptor.

1. The data yielded an unambiguous Scatchard plot
2. The dissociation constant ($K_d$) derived from the plot was in the $10^{-10}$M to $10^{-9}$M range
3. The unlabeled competitor used to derive the Scatchard data was diethylstilbestrol (DES)

Using these criteria, the tumor samples were divided into four groups, depending on the distribution of estrogen receptor. These groups were those with receptor in both soluble and pellet fractions (+/+); those with receptor in the soluble fraction only (+/0); those with receptor in the pellet fraction only (0/+); and those with no receptor in either fraction (0/0). The results are shown in table 1.

It was interesting to note that if the receptor status had been determined purely on competition at $10^{-9}$M (the so-called one-spot assay), then eight patients would have been +/+, eight patients +/0, and four patients 0/+. Diethylstilbestrol was used as competitor for these one-spot assays, so it is unlikely that the binding component was plasma steroid-binding globulin. However, it was impossible to construct a Scatchard plot for any of these biopsies from the data. Therefore, whatever the nature of this binding component, its affinity for estrogen was considerably lower than that of any of the natural cellular estrogen receptors.

TABLE 1.—Estrogen receptor distribution in 72 tumor biopsies

| Tumor | No. of biopsies | | | | |
|---|---|---|---|---|---|
| | Total | Receptor status | | | |
| | | +/+ | +/0 | 0/+ | 0/0 |
| Colorectal carcinoma | 51 | 1 | 1 | 1 | 48 |
| Malignant melanoma | 7 | 0 | 0 | 0 | 7 |
| Stomach carcinoma | 2 | 0 | 0 | 0 | 2 |
| Kidney carcinoma | 5 | 0 | 0 | 0 | 5 |
| Bladder carcinoma | 4 | 0 | 0 | 0 | 4 |
| Ureteric carcinoma | 2 | 0 | 0 | 0 | 2 |
| Retroperitoneal sarcoma | 1 | 0 | 0 | 0 | 1 |

## TAMOXIFEN THERAPY

A total of 30 patients, each of whom was attending the Clinical Oncology Clinic, were entered in this study. Twenty-nine had previously been treated with one or more combination chemotherapy regimens. Each patient had advanced, measurable disease, and each was treated with tamoxifen (20 mg twice daily). All patients received tamoxifen as their sole treatment during the study, and treatment was continued for at least 6 weeks unless obvious progression occurred earlier. Of the nonevaluable patients, three were withdrawn from the study because of side-effects and two died within 6 weeks, before followup assessment had been made.

The results of therapy in the evaluable patients are shown in table 2. Four of the malignant melanoma patients had tumor samples assayed for estrogen receptor and all four were negative for both cytoplasmic and nuclear receptors.

## DISCUSSION

Despite some earlier reports that an estrogen-binding component could be measured in a relatively high proportion of patients with some nonhormone-dependent cancers (6,7,8,10) the data presented here show that, under the strict assay criteria adopted, estrogen receptor of the type found in normal endocrine tissue and in breast cancer occurs only very rarely. Of the biopsies from 72 patients examined, only three had any cellular estro-

TABLE 2.—Response to tamoxifen therapy in patients with advanced, measurable disease

| Tumor | No. of patients— | | | |
|---|---|---|---|---|
| | Entered | Evaluable | With response | With progression |
| Malignant melanoma | 14 | 14 | 0 | 14 |
| Colorectal carcinoma | 7 | 5 | 0 | 5 |
| Stomach + esophageal carcinoma | 5 | 3 | 0 | 3 |
| Kidney carcinoma | 3 | 3 | 0 | 3 |
| Prostatic carcinoma | 1 | 0 | 0 | 0 |

gen receptor, and only one of these had both cytoplasmic and nuclear receptor characteristics of hormone-dependent tissue. One possible explanation of this discrepancy is that the use of one-spot assays can lead to false positives because of the presence of other proteins that bind estradiol with lower affinity. Or, there may be a separate estrogen receptor in these tissues which has different physical properties (including lower affinity for estrogens) from receptor normally associated with endocrine tissue and response to hormone therapy. If this is the case, then it remains to be seen whether the presence of the lower affinity receptor reflects any degree of hormone dependence.

Diethylstilbestrol is strongly recommended as an unlabeled competitor to eliminate any possible contamination with plasma steroid-binding globulins. This precaution is essential in biopsies of, for example, colorectal carcinoma, because some of them contain a great amount of blood.

The complete lack of objective remission seen in the patients treated with tamoxifen is in keeping with the apparent absence of functional receptor from these tissues (although receptor data were available only for a small proportion of those patients actually treated with tamoxifen). As a precaution, it should be remembered that all these patients had very advanced disease and had already failed to respond to one or more chemotherapeutic regimens.

In conclusion, there is, as yet, little evidence to suggest that steroid receptor assays are of significant value in selecting treatment of nonendocrine tumors. However, studies of the response of such patients to antihormones may well be justified in view of the poor performance with chemotherapy and the relative lack of toxicity of tamoxifen and related drugs.

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# Use of Tamoxifen in Advanced Male Breast Cancer

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## SUMMARY

**Tamoxifen citrate has been administered continuously at various dosages to 31 males with evaluable advanced breast cancer in 16 different centers. There were 15 complete or partial responders (48%) and five patients who were assessed to have achieved disease stabilization. In this series, there were responses in visceral-dominant (five of ten), bone-dominant (two of five), and soft tissue-dominant (eight of 15) disease. Response to tamoxifen appeared to be related to estrogen receptor status and to response to prior or subsequent endocrine therapy, although no firm conclusions can be drawn from such small numbers.**

[Cancer Treat Rep 64:801–804, 1980]

Cancer of the male breast is an uncommon disease. It is said to account for approximately 1% of all cases of breast cancer (1). The low incidence of the disease makes it difficult for any one investigator to achieve familiarity with the tumor and even more difficult to assess an individual treatment modality. Not surprisingly, therefore, we are unaware of any prospective phase II or III studies in male breast cancer. However, both Scheike (2) and Ribeiro (3) have reported the treatment of a large series of men attending their respective institutes.

The etiology, pathology, prognosis, and treatment of male breast cancer have also been reported by Meyskens et al (1) and Crichlow (4), who reviewed large numbers of studies to draw their conclusions. The practice of pooling data in an attempt to achieve adequate patient numbers has its dangers, such as incompatible response criteria between studies; however, such pooled data must suffice in the assessment of uncommon diseases and in the absence of controlled studies.

There have been many publications on the use of the antiestrogen tamoxifen (Nolvadex, property of Imperial Chemical Industries, Ltd.), as a palliative therapy for the treatment of advanced breast cancer in the female (5). We became aware that a number of investigators had achieved clinical responses with the drug in male breast cancer; the first publications appeared in 1978 (6–9). There are now 11 published case reports with no more than three cases in the largest series. We have been able to collect data on 21 more unpublished cases, 20 of which are assessable for response. The clinical data on these patients are presented and compared retrospectively to results obtained with standard therapies.

## MATERIALS AND METHODS

Case reports have been published on 11 patients (6–12).[4] These and the 21 unpublished cases are listed in table 1. Only 20 of these 21 patients were considered assessable for response since patient No. 22 died after only 1 day of therapy and was excluded from the analysis.

The patients' ages ranged from 45 to 88 years (mean, 66). Thirty patients received tamoxifen as their only anticancer agent; one patient received concomitant corticosteroid therapy. The tamoxifen

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[3]The following clinicians contributed details of unpublished case records to this review: W. H. Bond and Drs. T. B. Brewin; G. C. Escher; T. E. Godfrey; A. Goudsmit; D. T. Kiang; I. C. M. Paterson; G. G. Ribeiro; and E. Savlov.

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[4]Also: B. M. J. Cantwell, D. Tong, M. Minton, et al, personal communication; and G. N. Hortobagyi, A. Di Stefano, S. S. Legha, et al, personal communication.

TABLE 1.—Characteristics of patients studied

| Patient No. | Investigator | Age | Disease stage | Dominant site* | Estrogen receptor (ER) status† | Dose (mg/day) | Response | Duration (mos) |
|---|---|---|---|---|---|---|---|---|
| 1 | Ribeiro | 51 | III | ST | | 20 | CR | 10 |
| 2 | Ribeiro | 65 | IV | Lung | | 20 | PROG | — |
| 3 | Ribeiro | 77 | IV | Bone | | 20 | NC | 12 |
| 4 | Ribeiro | 81 | IV | Lung | | 20 | CR | 10 |
| 5 | Ribeiro | 63 | III | ST | + | 20 | PROG | — |
| 6 | Ribeiro | 53 | IV | Lung | − | 20 | PROG | — |
| 7 | Ribeiro | 45 | IV | ST | − | 20 | PROG | — |
| 8 | Ribeiro | 67 | IV | ST | − | 20 | PROG | — |
| 9 | Ribeiro | 50 | IV | ST | + | 20 | PR | 6 |
| 10 | Aisner | 47 | IV | Lung | +(PR+) | 30 | PR | 7 |
| 11 | Brewin | 84 | IV | ST | | 20 | PR | 12+ |
| 12 | Jeffreys | — | IV | Bone | | — | PR | — |
| 13 | Abele | 55 | IV | ST | | 40 | CR | — |
| 14 | Cantwell | 66 | IV | Bone | | 20 | CR | 30 |
| 15‡ | Cantwell | 48 | IV | Liver | | 40 | PR | 12 |
| 16 | Cantwell | 70 | III | ST | | 20 | CR | 3 |
| 17 | Paterson | 78 | IV | Lung | | 40 | CR | 20+ |
| 18 | Paterson | 79 | III | ST | | 40 | NC | 18+ |
| 19 | Kiang | 77 | IV | Lung | | 40 | NC | 8 |
| 20 | Kiang | 48 | IV | Bone | | 40 | PROG | — |
| 21 | Godfrey | 58 | IV | Lung | | 20 | PROG | — |
| 22 | Escher | 58 | IV | Bone | | 20 | — | — |
| 23 | Sakai | — | — | — | | 20 | PROG | — |
| 24 | Morgan | 65 | IV | ST | | 40 | PROG | — |
| 25 | Morgan | 70 | IV | Lung | | 20 | PROG | — |
| 26 | Bond | 83 | III | ST | | 40 | NC | — |
| 27 | Bond | 80 | III | ST | | 40 | PROG | — |
| 28 | Bond | 72 | III | ST | | 40 | CR | 4+ |
| 29 | Hortobagyi | 77 | IV | ST | | 20 | PR | 6 |
| 30 | Savlov | 88 | IV | ST | +(PR−) | 20 | CR | 7.5 |
| 31 | Goudsmit | 53 | IV | Lung | +(PR+) | 20 | PR | 1+ |
| 32 | Hortobagyi | 62 | IV | Bone | | 20 | NC | 5+ |

*ST = soft tissue.
†+ = positive; − = negative.
‡Received concomitant prednisolone therapy.

dosage ranged from 20 to 40 mg/day. All patients were said to have objectively assessable disease, although the criteria used for response were the investigator's own or an accepted method, and not standardized across the review. In this study, however, a complete remission (CR) is defined as disappearance of all clinical signs of disease for at least 1 month; partial response (PR) as > 50% decrease in measurable or evaluable lesions, with no progression at other sites or new lesions appearing, maintained for 1 month; and no change (NC) as < 50% disease regression or stabilization. Disease progression (PROG) was a measurable increase in size of lesions or the appearance of any new disease.

## RESULTS

### Efficacy

Of the 31 assessable patients there were eight with CR, seven with PR, and five with NC. Thus, the overall response rate (CR + PR) was 48% (15 of 31). The validity of quoting an overall response rate derived from data obtained largely from separate case reports is questionable, as some of the cases unsuccessfully treated may not have been reported to us.

The duration of response was 1+–30 months (mean, 9+ months). The five NC patients remained in disease stabilization for a mean of 10+ months. Table 2 shows the response rate in relation to dosage level. It can be seen that, as with female breast cancer, responses are observed at each dosage. Analysis by dominant disease site shows that five of ten (50%) patients with visceral disease, two of five (40%) with bone disease, and eight of 15 (53%) with soft tissue disease were classified as achieving CR or PR. Four other patients showed only subjective responses (13%).

Estrogen receptors (ER) were measured in eight tumors. Four of five patients with ER+ tumors responded and none of the three with ER− tumors showed remission.

TABLE 2.—Response to therapy by dose

| Total daily dose (mg) | Total No. of patients | Response | | |
|---|---|---|---|---|
| | | CR + PR (% total) | NC | PROG |
| 20 | 19 | 9 (45%) | 2 | 8 |
| 30 | 1 | 1 | — | — |
| 40 | 10 | 4 (40%) | 3 | 3 |
| Unknown | 1 | 1 | — | — |

Of the three responders to previous orchiectomy, two were classed as NC, and one as CR; the two patients who failed to respond to orchiectomy later failed to respond to tamoxifen therapy. Prior cytotoxic therapy failed to halt the disease in four men who were subsequently classified as achieving CR, PR, NC, and PROG, respectively, on tamoxifen. Two responders to chemotherapy later failed to respond to tamoxifen.

Two tamoxifen responders subsequently achieved remission on ablative endocrine therapy, after relapse. Two who failed to respond to tamoxifen also failed to respond to subsequent diethylstilbestrol (DES) therapy; one of these patients had an ER+ tumor.

### Side Effects

As with female patients, side effects in males were few. One patient was removed from tamoxifen therapy due to gastrointestinal intolerance and bone pain. Two other patients tolerated a dosage reduction from 30 to 20 mg daily after mild leukopenia developed. One man developed hypercalcemia, which resolved after withdrawal of tamoxifen and did not recur on reintroduction of the agent.

## DISCUSSION

Castration is the traditional treatment of choice for metastatic male breast carcinoma (13). This surgery yields high response rates (45% and 68% in two studies) (14,15), which may be prolonged. Adrenalectomy is also thought to be effective, but there are few reported cases (14,16–18).

The role of additive hormonal therapy is poorly defined (19). Ribeiro (20) reported a 38% response rate to DES in a retrospective review of 55 cases treated at one institute; the median remission was 7 years. However, no remissions were seen in patients with bone metastases. Cytotoxic agents have some efficacy (17,21), although the sparsity of data makes assessment difficult.

This report confirms the impressions of a number of investigators; tamoxifen is effective in treating male breast cancer and produces few side effects. The overall response rate in this small review is similar to that produced by other endocrine modalities in unselected cases. The duration of the responses cannot be accurately assessed at this early stage. Tamoxifen was effective in soft tissue, pulmonary, and osseous disease, as well as in one case with hepatic involvement. This patient also received concomitant corticosteroid therapy. These data compare favorably with Ribeiro's data on DES, which show responses only in patients with soft tissue and lung disease.

Tamoxifen is thought to act by competing with endogenous estradiol for the high-affinity cytoplasmic receptor protein. It may also have indirect effects, mediated via the pituitary-hypothalamic-gonadal axis in both males and females. In the small numbers of tumors for which estrogen receptor protein was measured, there is a suggestion that the assay may be helpful in defining patients likely to respond or to fail to respond to tamoxifen therapy.

The overall response rate to tamoxifen in > 1000 women with advanced breast cancer was 32% (5). Thus, a response rate of 48% in 31 males is at least equivalent to the female response rate. These data suggest that tamoxifen has a place in the therapy for advanced breast cancer in men and is worthy of further evaluation.

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# Treatment of Advanced Endometrial Adenocarcinoma With Tamoxifen

**Kenneth D. Swenerton**[1,2,*]

Advanced or recurrent endometrial adenocarcinoma is a less common clinical problem than breast carcinoma of similar extent. It is then not surprising that therapeutic advances have lagged in the former disease. However, as data accumulate, intriguing therapeutic similarities between the two are becoming apparent.

Several recent reports (1–6) of cytotoxic chemotherapy regimens in endometrial adenocarcinoma have shown a spectrum of drug activity paralleling that observed against breast carcinoma. Moreover, the prognostic factors influencing the quality of response to cytotoxic drugs seem similar (1,7).

Developing concepts of cytosolic steroid receptor function suggest that hormonal treatment analogies may also be expected. Breast carcinoma is unresponsive to hormonal treatment if steroid receptors are absent from the cytosol (8), but frequently responds to the antiestrogen, tamoxifen, if estrogen receptor (ER) is present (9). The cytosol of both malignant and nonmalignant endometrial tissue contains measurable ER (10), and the regulation of levels of ER appears to be similar in normal and neoplastic tissue (11). Tamoxifen has been shown to inhibit the binding of estradiol to uterine ER (12,13). If ER in malignant endometrial tissue has functional potential, then tamoxifen could block the proliferative stimulus of ambient estrogen. We therefore hypothesized that tamoxifen might produce clinically useful responses.

This report details our favorable experience in assessing the effectiveness of tamoxifen in advanced endometrial carcinoma and includes preliminary data reported elsewhere (14).

## MATERIALS AND METHODS

The trial was initiated in March 1978. Eligibility criteria required a confirmed histologic diagnosis in patients demonstrating progressive disease not suitable for established surgical, radiotherapeutic, or hormonal treatment. There were no age or performance status limitations. All patients had to have discontinued previous therapy at least 4 weeks before entering the study and have recovered from prior treatment toxicity. Measurable or evaluable disease had to be present.

Pretreatment evaluation included a history and physical examination, with particular attention to documenting measurable disease and estimating, retrospectively, the response to prior therapy. Standard hematologic and biochemical parameters were determined, and appropriate radiographic and imaging studies performed. Tumor steroid receptor data were not available. Followup was maintained at 4-week intervals to assess patient status, disease extent and response. Tumor grade was assigned as follows: *grade I*—highly differentiated adenocarcinoma; *grade II*—differentiated adenocarcinoma with partially solid areas; *grade III*—predominantly solid or entirely undifferentiated carcinoma.

Response criteria were based on those suggested by the International Union Against Cancer (UICC) (12): *complete remission (CR)*—disappearance of all clinical evidence of disease for a minimum of 4 weeks in an asymptomatic patient; *partial remission (PR)*—$\geq$ 50% decrease in the sum of the product of the diameters of measured lesions, without simultaneous increase in the size of any lesion or the appearance of new lesions; *stable disease*—steady state of response less than PR or lack of evidence of progressive disease for a period of at least 8 weeks; *progressive disease*—unequivocal increase of at least 25% of the size of any measured lesion or the appearance of any new lesion. The quality of response was further measured by the time to progression—the interval from the start of therapy until progressive disease was evident.

Tamoxifen was administered in an oral dose, 10 mg twice daily, and continued indefinitely in patients achieving remission or stable disease status.

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[2]The author wishes to acknowledge the secretarial assistance of Barbara McGaughey in preparing the manuscript and the collaboration of the following physicians in treating certain patients reported here: Dorothy Shaw; Wah Mei Chan; Malcolm Munro; Peter McComb; and Gerald W. White.

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Patients were removed from study if they developed progressive disease.

In an effort to achieve further palliation, some patients were subsequently treated with alternative hormonal therapy consisting of a combination of 10 mg of tamoxifen twice daily and 40 mg of megestrol acetate four times daily.

## RESULTS

Twelve patients have been entered in the study; two of these were entered too recently to be assessed.

The 10 evaluable patients demonstrated the following pretreatment characteristics. The age at initial diagnosis ranged from 47 to 71 years, with a median of 59 years. Primary therapy consisted of surgery plus radiotherapy in seven patients, surgery alone in two, and radiotherapy alone in one. The disease-free interval ranged from no interval to 110 months, with a median of 37 months. All patients had had prior therapy for recurrent disease; each patient had received prior progestational therapy in the form of medroxyprogesterone acetate at a continuous oral dose of 100 mg daily. Nine of the patients had received prior cytotoxic chemotherapy with cyclophosphamide; three of these also received doxorubicin and two 5-fluorouracil.

Ages at entry in this study ranged from 52 to 80 years, with a median of 65. The major site of disease at the beginning of the study was intrathoracic in five patients, pelviabdominal in four, and both sites in one.

## CASE REPORTS

Two patient histories are summarized for illustrative purposes.

*Patient 1.*—A diagnosis of clinical stage I, grade II endometrial adenocarcinoma was made in a 71-year-old woman. Primary therapy consisted of two intracavitary radium insertions, followed by hysterectomy and bilateral salpingo-oophorectomy. Following this treatment, she remained free of evident disease for 110 months. No adjuvant therapy was given. Disease recurred, with a mass evident

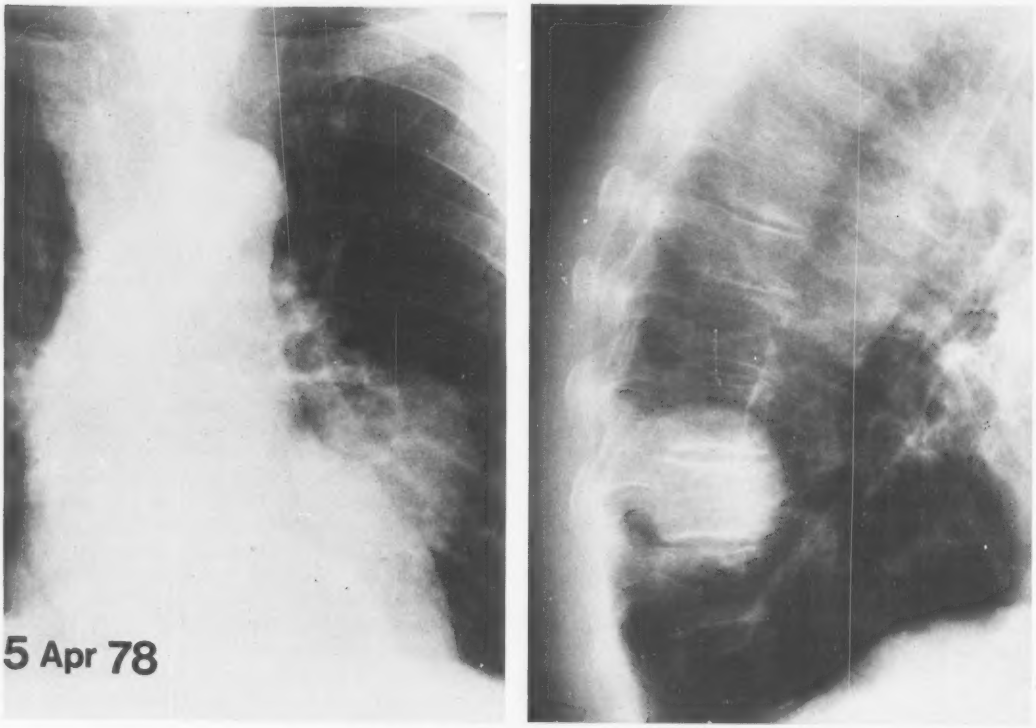


FIGURE 1.—Patient 1: radiographic findings when tamoxifen therapy was initiated.

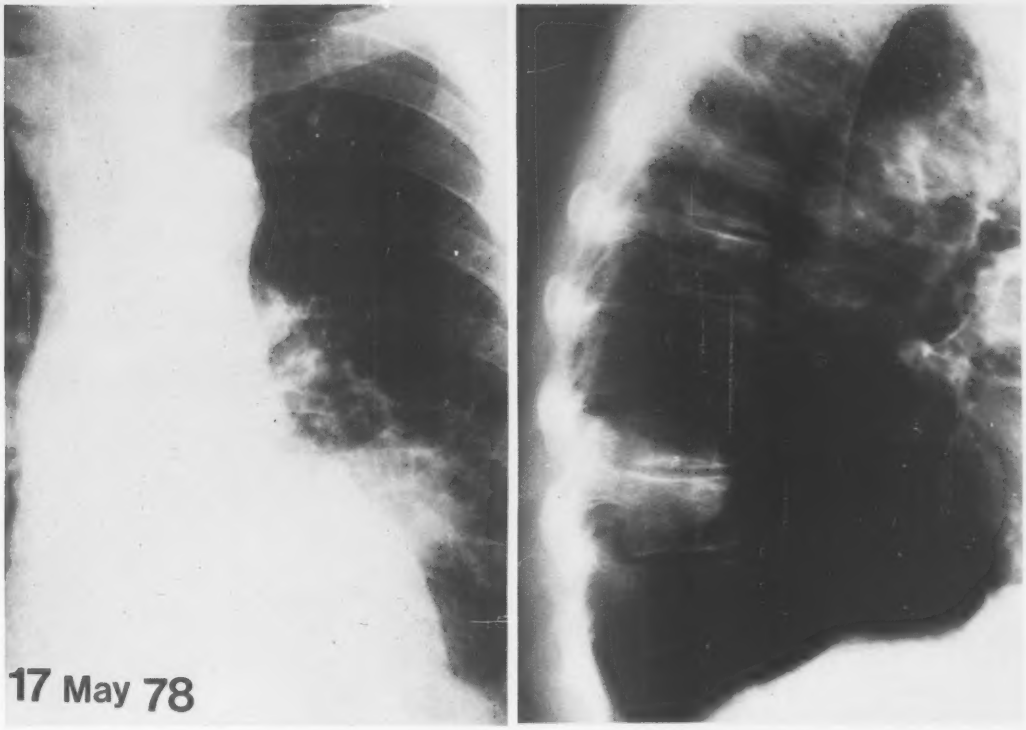


FIGURE 2.—Patient 1: radiographic findings at Wk 6 showing improvement (PR).

in the lung, and treatment was initiated with medroxyprogesterone acetate (100 mg daily continuously) and cyclophosphamide (150 mg/m$^2$ daily for 5 days each month). Less than a PR was achieved; it lasted 10 months. Loss of this primary response was evidenced by increasing size of the lung metastasis and the development of a cough. The radiographic findings at the time tamoxifen was initiated are shown in figure 1. Radiographic and symptomatic improvement was evident by Week 6 of the treatment (fig 2), with PR being achieved. Relapse was apparent by Week 34, and tamoxifen was discontinued. After tamoxifen was discontinued, the tumor continued to grow and, by Week 48, the patient showed increasing cough, shortness of breath, and hemoptysis. At Week 54 (fig 3), tamoxifen (10 mg twice daily) plus megestrol acetate (40 mg four times daily) was initiated, and symptomatic improvement was evident by Week 54. The lesion continued to decrease in size; the ongoing response at Week 60 is shown in figure 4.

*Patient 2.*—Clinical stage II, grade III endometrial adenocarcinoma was diagnosed in a 50-year-old woman. Total hysterectomy and bilateral salpingo-oophorectomy were performed and revealed tumor involvement of the adjacent bowel. Subsequent to surgery, external-beam pelvic radiotherapy was delivered to a central dose of 3125 rads in 35 fractions. The patient remained well for 58 months when recurrent disease was discovered in the abdominal wall. Medroxyprogesterone acetate (100 mg daily) was given, with an objective response lasting 32 months. At relapse, cyclophosphamide was administered without benefit. At entry in this study, the patient complained of diarrhea, intermittent intestinal bleeding, and progressive weight loss (from 61 to 38 kg). Investigation revealed multiple intra-abdominal and hepatic metastases and the previously evident abdominal wall mass. Clinical data are summarized in table 1. After 7 weeks of treatment, the patient noticed resolution of intestinal bleeding, and the abdominal wall mass

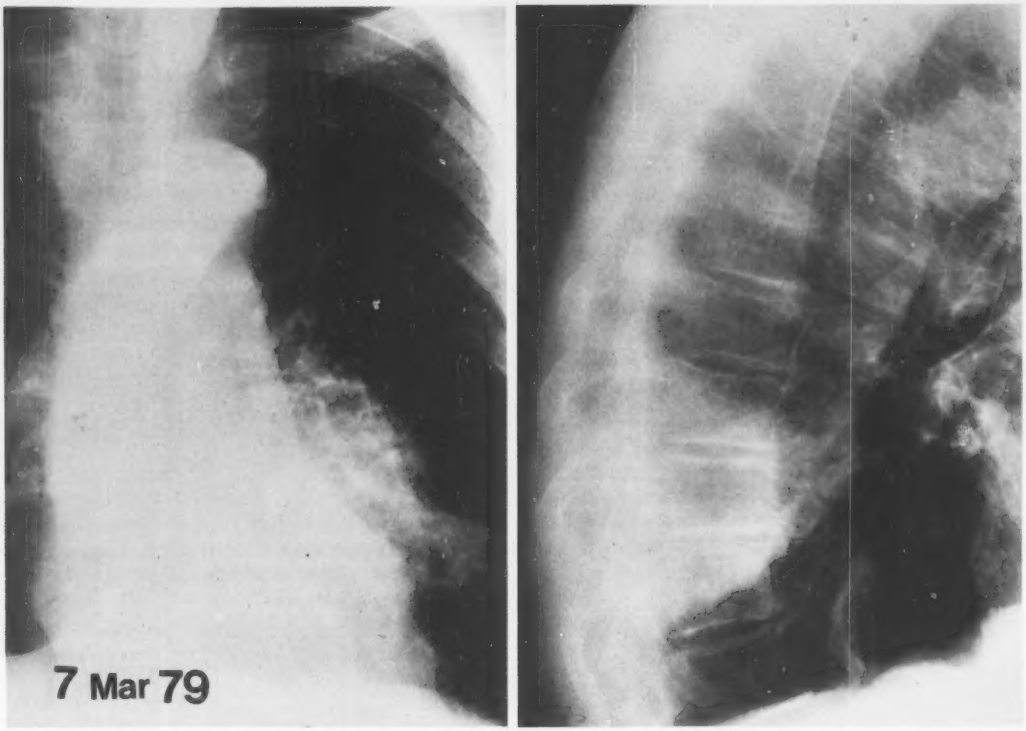


FIGURE 3.—Patient 1: radiographic findings at Wk 54 when tamoxifen + megestrol acetate therapy was instituted.

had decreased in diameter. The absolute granulocytosis, and elevated alkaline phosphatase (Alk P), lactic dehydrogenase (LDH), and carcinoembryonic antigen (CEA) values improved. There was complete resolution of diarrhea by Week 11. The response to tamoxifen was classified as stable disease (response < PR). Symptoms began to recur by Week 31. At Week 32, megestrol acetate was added to the tamoxifen regimen, but the disease continued to progress and death occurred at Week 39.

Overall, one patient achieved CR, two had PRs, two had stable disease (including one response less than PR), and five had disease progression. Time to response was 7 weeks, 6 weeks, and 8 weeks respectively. The likelihood of response appeared to be a function of tumor grade, as shown in table 2.

Allowing that patient numbers are small, the durability of response (as measured by the time to progression for patients with CR, PR, and stable disease) correlated with tumor grade and disease-free interval. All responding patients had disease-free intervals of > 2 years (table 3).

The correlation between response to prior progestational therapy and that achieved with tamoxifen is shown in table 4. One patient who demonstrated no benefit from prior progestin therapy achieved CR with tamoxifen.

There were no observed prognostic effects of age, predominant site of disease, or response to prior cytotoxic therapy on tamoxifen response.

Five of the eight patients removed from the study went on to "tertiary" hormonal therapy, with tamoxifen plus megestrol. Of this group, one patient's subsequent response cannot yet be determined. Of the remaining four patients, three had initially shown some response to tamoxifen (two PR, one stable disease), and one had progressed primarily while receiving tamoxifen. These data are summarized in table 5.

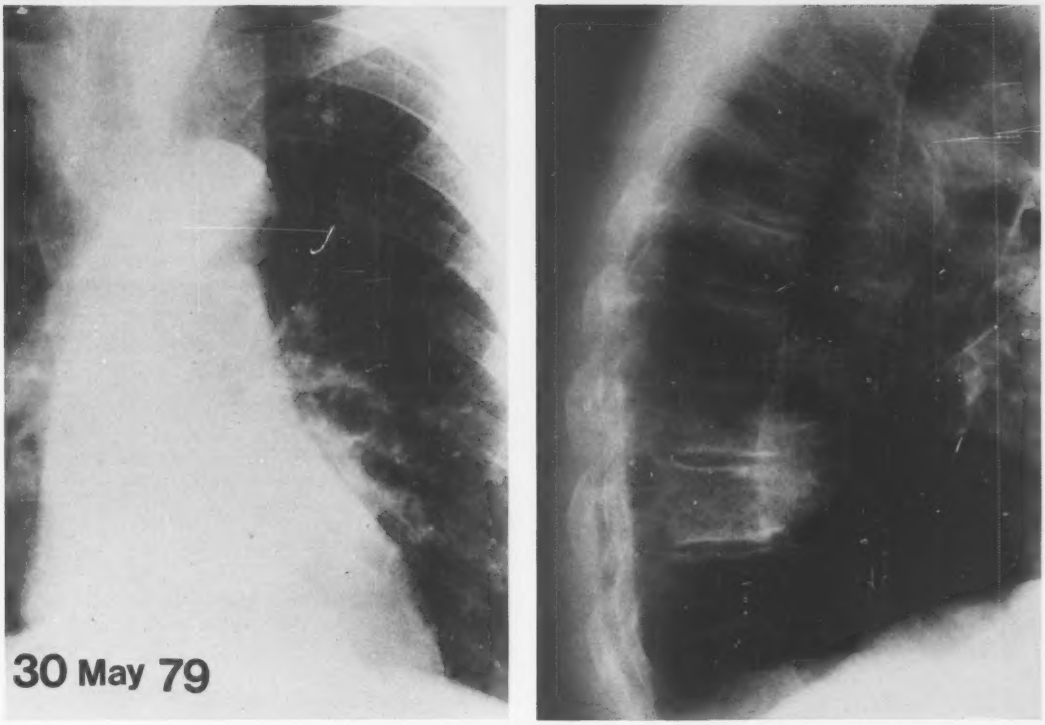


FIGURE 4.—Patient 1: radiographic findings at Wk 60, showing decreased lesion size and ongoing response.

TABLE 1.—Clinical data for patient 1

| Time (wk) | Hemoglobin (g/100 ml) | Wbc count (cells × $10^{-3}$) | CEA (n ≤ 5) | Alk P (xn̄)* | LDH (xn̄)* | Tumor (cm)† | PS‡ |
|---|---|---|---|---|---|---|---|
| 0 | 12.0 | 49.2 | 13.0 | 2.3 | 1.3 | 5.0 | 2 |
| 7 | 9.7 | 23.1 | 10.5 | — | — | 4.0 | 1 |
| 11 | 11.6 | 18.7 | — | — | — | 4.0 | 1 |
| 14 | 13.2 | 5.0 | 9.5 | 1.6 | 1.0 | 3.5 | 0 |
| 19 | 11.5 | 23.1 | 6.1 | — | — | 3.5 | 0 |
| 31 | 10.6 | 25.7 | 10.5 | 5.1 | 1.1 | 4.0 | 1 |

*xn̄ = times the upper limit of normal.
† = size of abdominal wall tumor.
‡PS = performance status according to Eastern Cooperative Oncology Group scale.

TABLE 2.—Response to tamoxifen as a function of tumor grade in 10 patients

| Tumor grade | No. of patients with— CR | PR | Stable disease | Disease progression |
|---|---|---|---|---|
| II | 1 | 2 | 1 | 2 |
| III | | | 1 | 3 |

TABLE 3.—Time to disease progression as a function of tumor grade and disease-free interval in 10 patients

| Tumor grade | Time (mos) to disease progression DFI > 2 yrs | DFI < 2 yrs |
|---|---|---|
| II | 12+, 12, 10, 7, 0 | 0 |
| III | 7 | 0, 0, 0 |

TABLE 4.—Response to tamoxifen as a function of response to prior progestin therapy in 10 patients

| Response to prior progestin* | No. of patients with— CR, PR, and stable disease | Disease progression |
|---|---|---|
| + | 3 | — |
| ? | 1 | 3 |
| – | 1 | 2 |

*+ = definite objective response; ? = equivocal or indeterminate response; – = no response.

TABLE 5.—Response to subsequent tamoxifen-megestrol therapy as a function of prior tamoxifen response

| Response to tamoxifen (5 patients)* | Response to tamoxifen + megestrol PR + stable disease | Disease progression |
|---|---|---|
| PR + stable disease | 2 | 1 |
| Disease progression | 0 | 1 |

*1 patient too early to assess.

## Toxicity

No incident of toxicity attributable to treatment was observed.

## DISCUSSION

Tamoxifen is an effective therapy for certain patients with advanced endometrial adenocarcinoma. The likelihood and quality of response may in part be predicted by tumor grade, length of disease-free interval, and response to prior progestational therapy. Lower grade (better differentiated) tumors in patients with longer disease-free intervals and who have responded to progestational agents have more favorable prognoses.

Our results are supported by the unpublished data of Broens et al. These Danish investigators report one CR, one PR, and one stable disease response in five previously untreated patients who were given an oral dose of 10 mg of tamoxifen three times daily.

There was a noteworthy lack of toxicity in our group of patients. One might predict, in view of the lower frequency of metastases to bone and marrow in this disease in contrast to breast carcinoma, that hypercalcemia will rarely be encountered. This lack of toxicity is a particular advantage in patients who are often elderly and have significant coexisting illness. The lack of toxicity would permit tamoxifen to be combined with cytotoxic chemotherapy with potential therapeutic advantage without additional toxicity.

The observation in one patient that tamoxifen produced a response in a tumor unresponsive to progestin supports the concept that antiestrogens and progestins exert their effect through separate, albeit functionally interrelated, steroid receptor mechanisms.

Some of our patients demonstrated prior responses to progestin, secondary responses to tamoxifen, and tertiary responses to the combination of tamoxifen and progestin. That sequential hormonal responses can be achieved extends the palliative potential of hormonal therapy in this disease. The clinical effectiveness of tamoxifen-progestin therapy was predicted by Sekiya and Takamizawa (16), who showed that these agents have a synergistic inhibitory effect on the colony-forming ability of cells derived from rat uterine adenocarcinoma in vitro. The mechanism of this synergism is unclear, but may be attributed to the induction of increased levels of progesterone receptor by tamoxifen. This effect, potentially re-inducing progestin sensitivity, was recently described by Robel et al (17) in human endometrial adenocarcinoma tissue in vivo.

With further knowledge of steroid receptor physiology and the development of techniques allowing more widely available and clinically practical receptor determinations, we should be able to modulate receptor levels to achieve more frequent and durable responses with hormonal treatment.

The parallels between endometrial adenocarcinoma and carcinoma of the breast provide an op-

portunity to explore treatment models developed for the more common disease. In particular, there is a need to determine the relative effectiveness of sequential and combination hormonal regimens, and to define more effective combination chemohormonal treatment regimens in advanced disease. As well, regimens found effective in advanced disease need to be studied as adjuvant therapy for patients at high risk for recurrent disease after they receive primary therapy.

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# Tamoxifen in Refractory Metastatic Carcinoma of the Prostate[1]

**John H. Glick,* Alan Wein, Kristin Padavic, William Negendank, David Harris, and Harvey Brodovsky[2,3]**

## SUMMARY

**Thirty-one patients with advanced metastatic carcinoma of the prostate were treated with the antiestrogen tamoxifen. Of these patients, 29 were refractory to prior hormonal manipulation with estrogens and/or orchiectomy and five achieved an objective response (partial regression or stable disease using National Prostatic Cancer Project criteria). Median survival of the responders was significantly longer than that of the nonresponders ($P < 0.05$). Two additional patients who had not previously received hormonal therapy both responded, for an overall response rate of 23% (seven of 31 patients). Toxicity was minimal and no tumor flares were reported. These preliminary results support the further clinical trial of tamoxifen in advanced cancer of the prostate.**

[Cancer Treat Rep 64:813–818, 1980]

Hormonal therapy using orchiectomy or estrogen therapy remains the well-established standard approach to the management of metastatic adenocarcinoma of the prostate. Although the data from the Veterans Co-Operative Urologic Research Group have challenged the ability of immediate versus delayed hormonal therapy to increase survival in patients with stage D disease (1), and although objective response of measurable disease other than serum acid phosphatase is uncommon (2), there is frequently a marked, subjective benefit with pain relief and improved performance status after hormonal manipulation.

The management of patients with advanced carcinoma of the prostate who are unresponsive to either orchiectomy or estrogen therapy, or who relapse after an initial subjective or objective response to hormonal treatment, remains controversial. Survival after relapse from initial endocrine manipulation approaches only 50% at 6 months (3). Nesbit and Baum (4) reported that patients who have previously undergone orchiectomy had only a 17% chance of responding to estrogens when relapse occurred, and only 36% of the patients who failed to respond to estrogen therapy derived benefit from orchiectomy. However, their response criteria were not well-defined, and the authors noted that the benefit the patients experienced may have resulted from improved general care rather than from the hormonal treatment itself. Brendler and Prout (5) randomly selected patients with prior orchiectomy to receive placebo or stilbestrol. Some 30% had a subjective response to the drug, but virtually no objective responses were noted. Although adrenalectomy and hypophysectomy have been used to treat this refractory group of patients, there remains considerable controversy over the efficacy of these hormonal modalities (6).

Recently, the antiestrogenic agent tamoxifen has been introduced for the palliative treatment of advanced breast carcinoma. Tamoxifen is a nonster-

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oidal derivative of triphenylethylene and specifically binds to estrogen receptor sites in target tissue. Tamoxifen's role in the treatment of breast cancer is well-established (7,8), and it offers an attractive alternative to diethylstilbestrol because it has minimal toxicity. Furthermore, postmenopausal women with metastatic breast carcinoma who have responded to diethylstilbestrol, and then failed, can be crossed over to tamoxifen with secondary objective benefit (9). The reverse sequence of tamoxifen response with secondary estrogen response has also been observed. The mechanism by which advanced breast carcinoma responds to both an estrogen and an antiestrogen is unknown. This intriguing observation, and recent case reports (10,11) of tamoxifen-induced responses in prostatic cancer, led to this phase II trial of tamoxifen therapy for patients who were refractory to estrogens and orchiectomy.

## METHODS

Patients selected for this study had histologically confirmed metastatic adenocarcinoma of the prostate and were unresponsive to or in relapse after conventional hormonal therapy with estrogens or orchiectomy. At least 6 weeks must have elapsed since orchiectomy was performed. Two weeks must have elapsed since cessation of estrogen treatment. If, in the opinion of the investigator, orchiectomy or estrogen therapy were contra-indicated for cardiovascular or psychologic reasons, patients could be entered in this study without prior hormonal therapy. All patients had progressive disease at the time of entry in the study, according to the published criteria of the National Prostatic Cancer Project (NPCP) (12). Patients were also eligible if they had previously received cytotoxic chemotherapy and had failed to respond or had subsequently developed progressive disease.

All patients had to have either measurable or evaluable disease. Patients having the combination of documented bone metastases and an elevated acid phosphatase value were considered eligible. Patients were all symptomatic at the time of entry and had an expected survival of at least 12 weeks. Only patients with an Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 3 were entered. (Table 1 contains explanations of performance.) Written informed consent was obtained.

Tamoxifen was administered in oral doses of 10 mg twice daily for 12 weeks. Patients were examined at 2–4 week intervals for objective and subjective evaluation of tumor response. If, at 12 weeks, the patient had achieved a partial response or was objectively stable, then tamoxifen was continued until progressive disease was documented, and the drug was then discontinued. If, at 12 weeks, the patient had progressive disease, tamoxifen was discontinued. Response criteria are those of the NPCP (table 2). To be classified as having partial response or being objectively stable, a patient could experience no deterioration of subjective ancillary parameters, including pain, weight, or performance status. Time to progression was defined as the time from the initiation of tamoxifen to the documentation of progressive disease. Survival was defined as the time from date of entry in the study until death.

Although pain relief was not considered an end point in determining objective response, pain was evaluated utilizing a Pain Score and an Analgesic Score developed for this study (table 3). A patient could not be classified as an objective responder or as being objectively stable if there were any deterioration in the Pain or Analgesic scores. Improvement in pain was defined as a > 50% decrease in both the Pain and Analgesic scores.

TABLE 1.—Pretreatment data for 31 patients receiving tamoxifen

| Characteristic | No. of patients |
|---|---|
| Prior therapy | |
| Estrogens and orchiectomy | 17 |
| Estrogens alone | 12 |
| Chemotherapy | 10 |
| Radiotherapy | 20 |
| None | 2 |
| Elevated acid phosphatase | 31 |
| Elevated alkaline phosphatase | 21 |
| Abnormal metastatic bone survey | 31 |
| Performance status | |
| 0 (normal activity) | 1 |
| 1 (symptomatic, but ambulatory) | 11 |
| 2 (bedridden < 50% of time) | 15 |
| 3 (bedridden > 50% of time) | 4 |
| Hemoglobin (g/100 ml) | |
| > 12 | 6 |
| 10.0–11.9 | 16 |
| < 10.0 | 9 |
| Pain | |
| Receiving narcotics | 24 |
| Receiving nonnarcotic analgesics | 6 |
| Receiving no analgesics | 1 |

## RESULTS

Thirty-four patients with progressive stage D carcinoma of the prostate had been entered into this

TABLE 2.—NPCP response criteria

| Response | Criteria |
|---|---|
| Complete objective response | *a.* absence of any clinically detectable soft tissue mass which may include primary tumor<br>*b.* return of elevated acid phosphatase to normal level<br>*c.* recalcification of all osteolytic lesions if present<br>*d.* no evidence of progression of osteoblastic lesions if any are present<br>*e.* if hepatomegaly is a significant indicator, complete reduction in liver size and normalization of all pretreatment abnormalities of liver function |
| Partial objective response | *a.* a 50% reduction in measurable or palpable soft tissue tumor mass when present<br>*b.* return of elevated acid phosphatase to normal<br>*c.* recalcification of some osteolytic lesions if present<br>*d.* if hepatomegaly is a significant indicator, reduction in liver size and at least a 30% improvement of all pretreatment abnormalities of liver function<br>*e.* no increase in any other lesion and appearance of no new areas of malignant disease<br>*f.* no significant deterioration in weight (>10%), symptoms, or performance status (1 score level) |
| Objectively stable, all of the following | *a.* insufficient regression of primary indicator lesion to meet criteria previously mentioned<br>*b.* 25% increase in any measurable lesion<br>*c.* no significant deterioration in weight (>10%), symptoms, or performance status (one score level) |
| Objective progression (or relapse after adequate therapy), any of the following | *a.* significant deterioration in symptoms, decrease in weight or in performance status<br>*b.* appearance of new areas of malignant disease<br>*c.* increase in any previously measurable lesion (soft tissue and lung, exclude bone) by 50% in 2 perpendicular diameters<br>*d.* increase in acid of alkaline phosphatase alone is not considered an indication of progression; these should be used in conjunction with other criteria |

TABLE 3.—Subjective evaluation of pain

| Evaluation | Score | |
|---|---|---|
| Pain score* | | |
| Severity of pain | | Frequency |
| None | 0 | None |
| Mild | 1 | Occasional |
| Moderate | 2 | Part of day (less than half) |
| Severe | 3 | Most of day (more than half) |
| Disabling | 4 | Constant |
| Analgesic score† | | |
| Analgesic use | | Frequency |
| None | 0 | None |
| Nonnarcotic (eg, aspirin) | 1 | Once a day |
| Mild narcotic (eg, codeine) | 2 | More than once a day |
| Strong oral narcotic (eg, dilaudid) | 3 | Most of the day |
| Parenteral narcotic | 4 | Constant |

*Severity × frequency.
†Use × frequency.

study as of May 1, 1979. Three patients were inevaluable: one patient discontinued the drug after 7 days, although he had experienced no toxicity; one patient with an initial performance status of 3 died from progressive disease after 11 days of tamoxifen therapy; one died of an acute myocardial infarction on Day 22 of therapy. The latter patient had suffered two recent myocardial infarctions and was receiving digoxin, furosemide, and quinidine at the time of entry in the study. The median age of the 31 evaluable patients was 68 years (range, 51 to 84). The median time from the original diagnosis of prostatic carcinoma to the time tamoxifen was started was 3 years and 2 months.

Table 1 shows some characteristics of the 31 evaluable patients. All but two patients had previously been treated with estrogens or by orchiectomy. All orchiectomy patients had also received estrogens at some time. Both patients who had not previously received conventional hormonal therapy had a past history of severe cardiovascular disease. Ten patients had received prior cytotoxic chemotherapy either as single agents or in combination. All 31 patients had elevated acid phosphatase and abnormal metastatic bone survey findings at the time they entered the study. Nineteen patients were bedridden (ECOG performance status 2 or 3); 12 patients were ambulatory (performance status 0 or 1). At the time of entry in the study, 25 patients were anemic, and all but one patient was experiencing pain from metastatic disease (24 patients were receiving narcotics pretreatment).

Table 4 shows the response to tamoxifen in the 29 patients who had previously been treated by hormonal manipulation. Two patients achieved a demonstrated partial response, and three were classified as being objectively stable according to NPCP response criteria. Thus, a response rate of 17% (five of 29 patients) in refractory prostatic carcinoma was achieved with tamoxifen therapy. Of these five tamoxifen responders, three had had prior therapy with both estrogens and orchiectomy (two of whom had responded), and two had previously received estrogens alone (one with a documented response). Among responders, some evidence of objective and subjective benefit occurred within the first 4–6 weeks of therapy. As seen in figure 1, the median survival time of the responders has not been reached, but will exceed 48 weeks (three of five responders are still alive). The median survival of the nonresponders is 15.5 weeks (only five of 24 are still living). The median survival rate of the responders is significantly longer than that of the nonresponders at the $P < 0.05$ level (generalized Wilcoxon test). It is important to note that

TABLE 4.—Response to tamoxifen in 29 hormonally resistant patients

| Response | No. of patients | % |
|---|---|---|
| Partial remission | 2 | 7 |
| Objectively stable | 3 | 10 |
| Total response | 5 | 17 |
| Progression | 24 | 83 |
| Total | 29 | 100 |

the additional two patients who had not received prior hormonal or ablative therapy both responded (one partial remission and one objectively stable). Thus, 23% of the entire group of 31 patients responded to tamoxifen. Details of the seven responders are summarized in table 5.

Pain was evaluated according to the criteria listed in table 3. All three patients who achieved a partial response experienced substantial and prompt relief of pain. Of the four patients who were objectively stable, three had improved Pain and Analgesic scores; one patient's pain pattern did not change. Of 24 patients with progressive disease, four experienced significant pain relief for the 12 weeks of study.

Toxicity was mild and no obvious tumor flares were noted. No dramatic increase in bone pain was reported immediately after starting the tamoxifen regimen. One patient with a previous history of an acute myocardial infarction, who was experiencing angina and being treated with nitroglycerin when he entered the study, had an exacerbation of his angina at a time when his hemoglobin count was 6.3. His symptoms were relieved promptly by transfusion of packed rbcs. Tamoxifen therapy was continued in this patient for the full 12-week period without further exacerbation of the angina. Another patient had a previous history of two myocardial infarctions; he also was taking nitroglycerin for angina and had a hemoglobin count of 10.3 when he entered the study. He experienced a documented acute myocardial infarction after 18 days of tamoxifen therapy, at a time when his hemoglobin count was 8.3. This condition was thought to be unrelated to the tamoxifen and he continued on the medication for the full 12-week period without subsequent exacerbation of his cardiovascular status. One patient with an initial platelet count of 138,000/mm$^3$ developed thrombocytopenia to 48,000 cells/mm$^3$, but a bone marrow biopsy demonstrated extensive infiltration with carcinoma, and his platelet count did not improve when tamoxifen was stopped. Two patients experienced mild nausea without vomiting. No episodes of skin rash, hypercalcemia, or other endocrinologic dysfunction were noted.

TABLE 5.—Characteristics of patients responding to tamoxifen

| Patient No. | Age | Prior hormonal therapy | Objective response to tamoxifen | Subjective response | | | Time to progression (wks) | Survival (wks) |
|---|---|---|---|---|---|---|---|---|
| | | | | Pain | Weight | Performance status | | |
| | | | *Partial responses* | | | | | |
| 1 | 75 | Yes | Acid phosphatase → normal | Markedly improved | Stable | Stable | 20 | 88 |
| 2 | 63 | Yes | Acid phosphatase → normal | Completely relieved | Increased 10 kg | Improved | 37+ | 37+ |
| 3 | 72 | No | Measurable nodes and lung lesion decreased by > 50% | Improved | Stable | Improved | 23 | 34+ |
| | | | *Objectively stable responses* | | | | | |
| 4 | 51 | Yes | Acid phosphatase decreased by > 50% | Markedly improved | Increased 8 kg | Improved | 24 | 53 |
| 5 | 69 | Yes | Acid phosphatase decreased by > 25% | No change | Stable | Stable | 18 | 21+ |
| 6 | 73 | Yes | No change in bone scan or roentgenograms | Completely relieved | Increased 3.5 kg | Improved | 13+ | 13+ |
| 7 | 71 | No | Acid phosphatase decreased by > 50% | Improved | Increased 7 kg | Improved | 16 | 17 |

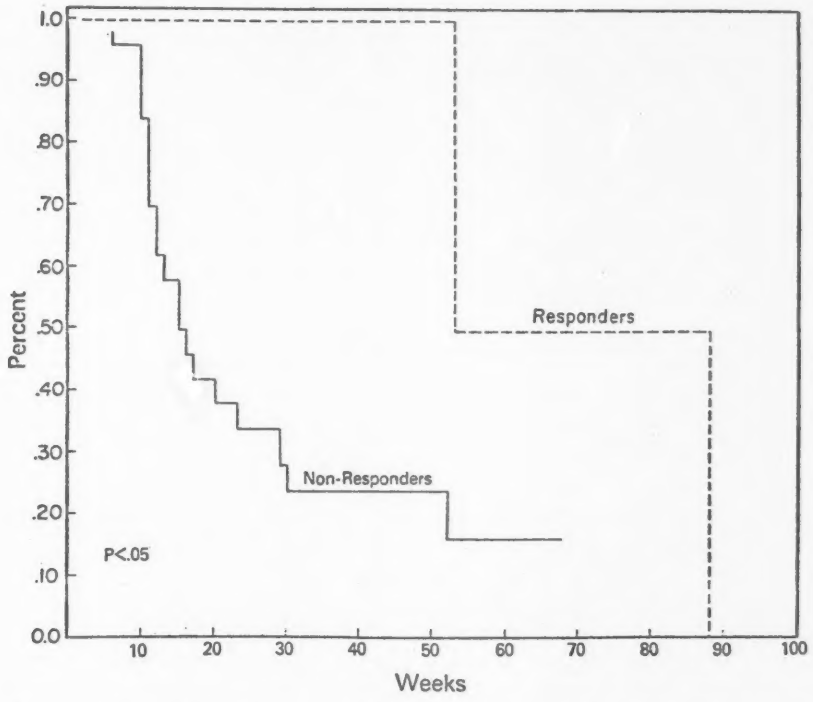


FIGURE 1.—Survival of the 29 patients receiving tamoxifen who were refractory to prior hormonal manipulation.

## DISCUSSION

This phase II clinical trial demonstrates that the antiestrogen tamoxifen is a potentially effective new hormonal agent for the treatment of metastatic carcinoma of the prostate. Five of 29 (17%) patients who were refractory to prior hormonal manipulation with estrogens and orchiectomy achieved an objective response on tamoxifen. Although the criteria for measuring response in advanced prostatic carcinoma are limited and controversial, we utilized the previously evaluated criteria of the NPCP to document objective responses. If objectively stable disease is an indicator of therapeutic effectiveness, then the data reported here support the continued investigation of tamoxifen. Although the mechanism of regression and symptomatic improvement of patients on tamoxifen therapy is not known, our partial remissions and objectively stable responses were accompanied by significant pain relief, increases in appetite and weight, and improved performance status. Most important, the median survival of the responders was significantly longer than that of the nonresponders. Subjective benefit was achieved in four of 24 patients with progressive disease who demonstrated significant pain relief with tamoxifen.

Two more patients, who had not previously received estrogen or ablative therapy because of medical contraindications, both responded to tamoxifen. The drug was well-tolerated and no patient was withdrawn from the study because of toxicity directly attributable to tamoxifen. No obvious tumor flares were noted.

Thus, an overall response rate of 23% in the 31 patients treated with tamoxifen was documented. We believe that these preliminary results support the further clinical investigation of tamoxifen in advanced carcinoma of the prostate and justify a trial of this agent in patients not previously treated with hormonal manipulation.

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# Estrogen and Progesterone Receptors and Tamoxifen in Malignant Melanoma

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## SUMMARY

**Tumor biopsies from 68 patients with malignant melanoma were assessed for estrogen receptor (ERc) binding, and 32 of these for progesterone receptor (PRc) binding. Twenty-five patients (37%) had ERc ≥ 5 fmols/mg protein; 14 patients (44%) had PRc ≥ 10 fmols/mg of protein. There was no significant difference between the sexes in relation to the presence or absence of ERc or PRc. The tumor ERc and PRc levels did not predict the survival of these patients. Seventeen patients were treated with tamoxifen; three female patients had objective responses. Two of the patients with complete response had ERc levels of 4.3 and 19 fmols/mg tumor cytosol protein, while the third patient had no detectable ERc.**

[Cancer Treat Rep 64:819–827, 1980]

The role of steroid hormones in the growth and spread of human malignant melanoma has long been a subject of considerable interest. Observations that suggest that these hormones may influence this disease include (*a*) the rarity of the incidence of malignant melanoma before puberty (1); (*b*) the bimodal peak incidence in females, coinciding with child-bearing years and menopause (2); (*c*) the adverse effect of pregnancy on stage II (regional node metastasis) melanoma (3); and (*d*) the studies by White (4), Nathanson et al (5), and Shaw et al (6), indicating longer survival rates in females than in males. Also, there is evidence indicating that malignant melanoma arises de novo or becomes worse following exogenous estrogen administration (1). In addition, recent studies (7) indicate that the incidence of malignant melanoma among women using oral contraceptives is higher than in women who do not use them.

Fisher et al (8) have reported the presence of estrogen receptors in 46% of the patients studied with malignant melanoma. Progesterone receptors were found in tumors of three of six patients (9), and in six of 27 patients (10) with melanoma.

This study reports that biopsy samples of tumors, in a substantial number of patients with malignant melanoma, contain appreciable levels of estrogen and progesterone receptors. The correlation between the presence of these receptors and the survival of the patients and the response of 17 patients to treatment with tamoxifen is also reported.

## MATERIALS AND METHODS

Tumor biopsies taken from 68 patients were assayed for estrogen receptor (ERc) binding and, in 32 cases in which the sample of tissue was sufficient, progesterone receptor (PRc) binding assays were also performed. Tumor samples were transferred to small bottles, immediately placed on ice, and transported to the laboratory within 30 minutes. The samples were then trimmed of surrounding adipose and necrotic tissues, washed with 0.9% saline, frozen in liquid nitrogen, and stored in a –80°F Revco freezer until examination, usually within 1–2 weeks of collection. A portion of the biopsy specimen was examined histopathologically to confirm the diagnosis and staging of the disease. The age of the patients ranged from 22 to 84 years (mean, 51.2; median, 52.5).

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TABLE 1.—ERc levels in melanoma patients treated with tamoxifen

| Patient No. | Sex | Age (yrs) | ERc (fmols/mg protein) |
|---|---|---|---|
| 1 | M | 58 | 3 |
| 2 | F | 53 | 11 |
| 3 | M | 49 | 154 |
| 4 | F | 44 | 19 |
| 5 | F | 50 | 2 |
| 6 | F | 42 | 29 |
| 7 | F | 67 | Not detectable |
| 8 | F | 66 | 4 |
| 9 | M | 58 | Not detectable |
| 10 | F | 50 | Not detectable |
| 11 | M | 72 | 15 |
| 12 | M | 42 | Not detectable |
| 13 | F | 76 | 19 |
| 14 | F | 61 | Not detectable |
| 15 | M | 51 | 8 |
| 16 | F | 36 | Not detectable |
| 17 | F | 47 | Not detectable |

For the determination of estrogen and progesterone receptors, the tissue was weighed and placed in 4–5 volumes of ice-cold buffer containing 10 mM tris, 1.5 mM EDTA and 0.5 mM dithiothreitol, pH 7.4 at 4°C (TEDG buffer). The cell-free preparation was made by homogenizing the tissue using a Polytron PT 10 ST (Brinkman Instruments, Inc, Westbury, NY) setting at 5; tissue was homogenized four times for 15 seconds each, allowing a 1-minute cooling interval between each burst. The homogenate was centrifuged at 105,000 × *g* at 4°C for 1 hour. The resulting supernatant (cytosol) was stripped of endogenous steroids by addition of 10% dextran-coated charcoal suspension in TEDG buffer, and vortexed intermittently for 2 minutes. The suspension was centrifuged at maximum speed of the IEC clinical centrifuge. All procedures were carried out at 4°C. The soluble cytoplasmic protein concentration was determined according to Lowry et al (11) and ranged from 6 to 8 mg/ml. The receptor assays were performed by Scatchard analysis of the binding data using estradiol 17-β and progesterone, and concentrations of these steroids ranging from 1 to 15 nM. The bound radioactivity was separated from the free steroid by dextran-coated charcoal. In this study, > 5 fmols/mg cytosol protein for ERc and 10 fmols/mg cytosol protein for PRc are considered positive values.

Using a four-stage classification system (12) for disease, one patient had stage I melanoma, 1 patient had stage II, 26 patients had stage III, and 40 patients had stage IV. There were 42 males and 26 females. Of 68 biopsies, two were obtained from the head and neck area, 18 from the trunk, one from the upper extremity, ten from the lower extremity, 12 from the viscera, 20 from the axilla, and five from the groin.

Seventeen patients with stage IV malignant melanoma, who failed protocols with established chemotherapeutic agents, were treated with 20 mg of oral tamoxifen twice daily. The age, sex, and ERc for each of these patients are shown in table 1.

## RESULTS

In the group of 32 patients who had both estrogen and progesterone assays performed, 16 had no detectable levels of either receptor; 12 of the patients were positive for ERc and PRc. Of the remaining four patients, two were positive for ERc only and two for PRc only. This association between the presence of estrogen and progesterone receptors in the same tumor specimen was highly significant ($P < 0.001$). Of 42 males, 13 had positive ERc values; of 26 females, 12 had positive ERc values. Thus, 37% of all the patients showed the presence of ERc. Progesterone receptors assayed in a total of 18 males showed eight patients with positive PRc, and assays in 14 female patients showed six (43%) with the presence of PRc. The difference in the incidence of positive ERc or PRc between the sexes was not significant.

Forty-three patients with no detectable ERc had a median survival of 21.3 months from the time of diagnosis of primary melanoma; 25 patients with positive ERc had a median survival of 33.6 months. Eighteen patients with negative PRc had a median survival of 20 months, and 14 patients with detectable PRc had a median survival of 23 months. The

differences were not significant in the median survival of patients, with or without the presence of ERc or PRc. There were also no significant differences in median survival, between ERc-positive and ERc-negative patients, estimated from the time of biopsy (6.4 vs 6.7 months).

Furthermore, no prognostic correlations could be identified according to ERc or PRc status, when subsets such as sex, stage of disease, or primary sites were considered.

Tumor specimens taken from different sites were assayed in four patients at various times. In the initial samples, values of 7, 4, and 19 fmols/mg protein were obtained for ERc in three of these patients. In the same patients, but in samples taken 3–12 months later, the values (fmols/mg) were 15, 3, and 0, respectively. In the fourth patient, the ERc was 27 fmols/mg protein and PRc was 16 fmols/mg protein in a recurrent primary tumor. Metastatic melanoma in one of his regional lymph nodes, biopsied the same day, was negative for ERc and PRc.

## DISCUSSION

The identification of estrogen receptors in malignant melanoma by Fisher et al (8) raised the possibility of hormonal manipulation in an identifiable subset of patients with disseminated melanoma. However, diethylstilbestrol therapy for advanced malignant melanoma in 18 patients produced objective regression in only two patients and neither had receptors in their tumor tissue (9). In an earlier report by Johnson et al (13), 11% of the patients with melanoma responded to 6α-methyl-pregn-4-ene-3,11,20-trione, a drug with antiestrogenic and weak glucocorticoid activity.

In the present study, 25 of 63 (37%) patients were found to have estrogen receptors. There was no significant difference between the sexes in the incidence of the presence of ERc or PRc. Moreover, no differences were observed relative to the biopsy site or tissues involved. The data were analyzed in various ways in an effort to identify prognostic implications associated with the presence or absence of ERc or PRc. No significant correlations could be identified between ERc or PRc status and the course of the disease in all of the patients studied or in various subsets. However, median survival, established from the time of diagnosis of primary melanoma, was longer by 1 year (although not statistically significant) in the patients with positive ERc. It remains to be seen whether this difference exists or is significant in a larger group of patients.

Fourteen of the 17 patients given tamoxifen demonstrated progression after a course of 1 month. Of the three remaining patients, two had a complete regression; and one had a $> 50\%$ objective re-

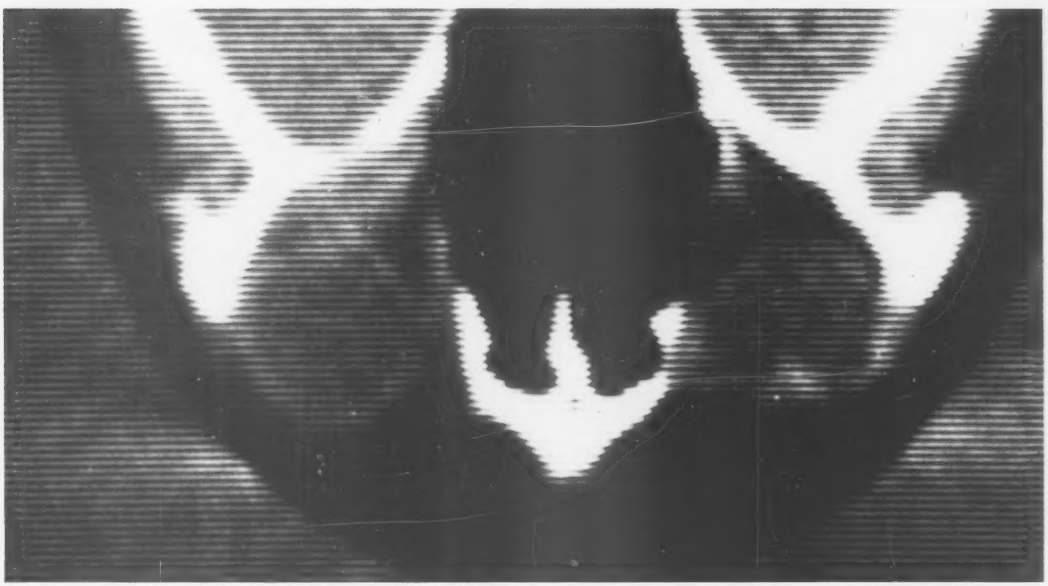

FIGURE 1.—Soft-tissue density behind the right eyeball.

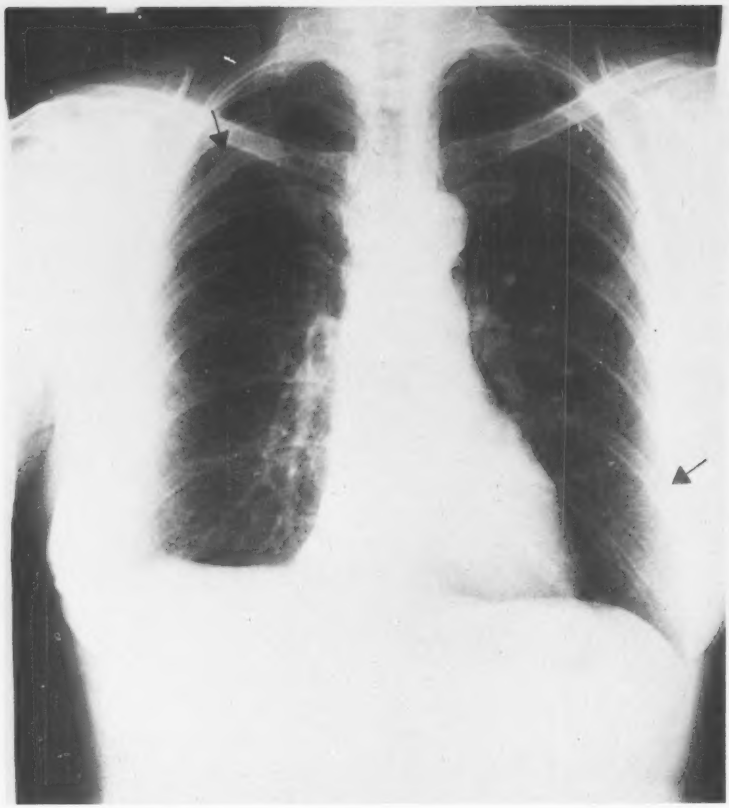

FIGURE 2.—Pathologic rib fractures.

sponse. Among the three responders, one patient (No. 8, table 1) had primary melanoma of the right leg excised in March 1977; a local recurrence was excised in January 1978. In April 1978, she had a pathologic fracture of the left femur treated with internal fixation and the diagnosis of metastatic disease was established histologically. In September 1978, she had multiple subcutaneous nodules, sixth-nerve paralysis (diplopia on lateral gaze), exophthalmos of the right eye (fig 1), and pathologic rib fractures (fig 2). She started to receive tamoxifen and, within 3 months, there was complete regression of the subcutaneous nodules and pulmonary metastases. There was evidence of healing of all pathologic fractures (fig 3), and she became asymptomatic (fig 4). Her complete regression lasted about 1 year, and then in November 1979, an intra-abdominal mass was found. During an exploratory laparotomy, a single mass in the right colon was removed with a right hemicolectomy. In the resected recurrent melanoma, the level of ERc was determined to be 3 fmols/mg protein.

Patient 13 (table 1) had malignant melanoma of the left eye removed by enucleation in 1976. A large intra-abdominal mass in the greater omentum was removed in April 1979; the level of ERc in this tumor was 19 fmols/mg protein. She also had pleural effusion and a nodule in the left lung (fig 5). One month following treatment with tamoxifen, the pleural effusion and the nodule disappeared (fig 6). She had a recurrence of the intra-abdominal melanoma 3 months later; this lesion was excised and was negative for ERc.

Patient 17 (table 1) had primary melanoma in the left ankle, Clark's level V, 10 mm thick. This area was widely excised and elective ilio-inguinal dissection and hyperthermic perfusion of the left leg with melphalan was given in May 1978. In August

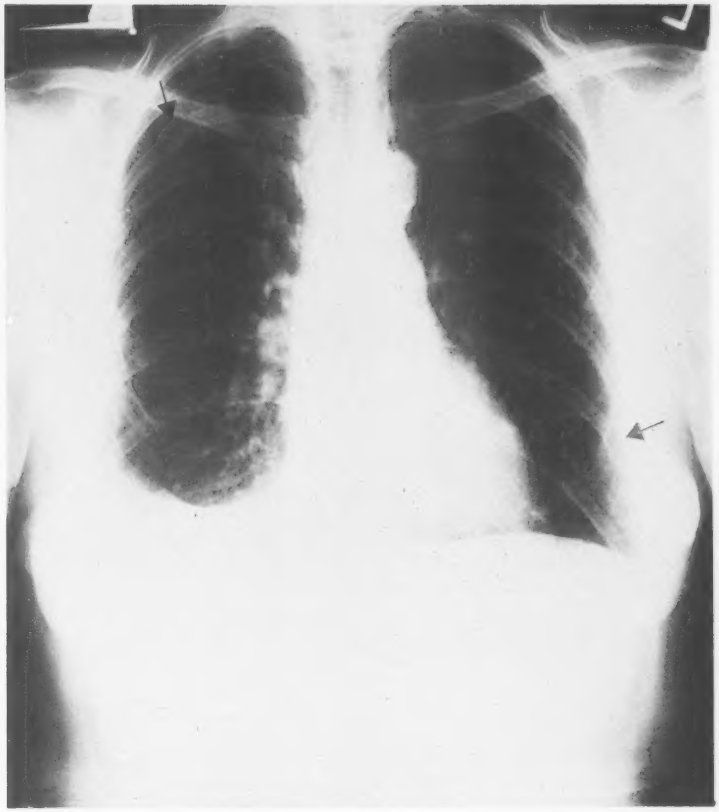

FIGURE 3.—Evidence of healing of pathologic rib fractures after treatment with tamoxifen.

FIGURE 4.—Soft-tissue density behind the right eyeball is no longer present.

1979, she had multiple subcutaneous nodules and was given a course of chemotherapy with 80 mg/m² of BCNU, 10 μg/kg of actinomycin-D, and 1 mg/m² of vincristine. New subcutaneous tumor nodules, however, continued to appear every week and she started to receive tamoxifen. One month later, two tumor nodules disappeared and the others had decreased by > 50%. Two months after beginning treatment, four more nodules had disappeared; one remaining nodule remained stable. One month later, an apparently new nodule (0.5 cm) was felt in the occipital area.

Three of the 19 patients treated with tamoxifen had objective regression of their tumors. The common characteristic of these patients was that they were all females. The two patients with complete regression had detectable estrogen receptor levels in tumor biopsies, and the patients with partial objective response had negative ERc. However, the fact that some of the subcutaneous nodules of the last patient regressed completely, and one remained unaffected after initial regression, may suggest a heterogeneity of tumor response. Similarly, patient 13 (table 1), had ERc levels of 19 fmols/mg protein in the initial tumor biopsy; the tumor that recurred had a negative ERc value. The variability observed with regard to the presence of ERc in two of three other patients indicates heterogeneity in melanoma cell populations and suggests that multiple specimens be examined, from each patient when possible, to assess the presence of ERc for greater prognostic significance. The lack of a definite correlation in this study between ERc status and response to tamoxifen is possibly due to inadequate sampling of tumors or to an alteration of the receptor status as the tumor progresses.

Tamoxifen, administered after patients fail to respond to standard chemotherapeutic agents, appeared moderately effective in this study. Our initial observations suggest that estrogen and progesterone receptor levels in melanoma may have prognostic implications in certain subsets of patients with the disease. However, further work is needed to identify a way to select the patients most likely to benefit from hormonal therapy.

FIGURE 5.—Nodule in the left lung and left pleural effusion.

FIGURE 6.—Nodule in the left lung and pleural effusion no longer apparent.

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